Investigating the bone-muscle interaction during growth and development in children

by

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ABSTRACT

INVESTIGATING THE BONE-MUSCLE INTERACTION DURING GROWTH AND DEVELOPMENT IN CHILDREN

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The purpose of this dissertation was to assess functional changes in the muscle-bone unit during normal growth and maturation in peri-pubertal children, and determine if changes in muscle strength are directly related to changes in bone properties.

The first part of this work was a systematic review of literature on the effect of physical activity on bone development in children. It was found the best *time* to see large improvements in bone properties may be during the peri-pubertal years. It was not clear the best *type* of activity, nor which loading characteristic, should be utilized. This led to the second part of this work, where a non weight-bearing bone, the radius, was investigated in order to separate the influence of muscle properties on bone from ground reaction forces.

Children and adolescents (n=172), between the ages of 8-16 years, were examined over a 2-year period. Measurements of somatic maturity, anthropometry, grip strength, bone properties (reflected by speed of sound (SOS)), physical activity (accelerometery), nutrition (24-hour recall), and bone resorption (NTX) were taken. Materials and procedures were identical between studies allowing for both a cross-sectional and longitudinal examination of the muscle-bone unit. Cross-sectionally, results demonstrated relative grip strength, maturity, dietary calcium and NTX explained 21% of the variance in radial SOS ($p<0.05$). Calcium intake was found to be a significant predictor only after NTX was accounted for, suggesting its effects on the muscle-bone unit may be modulated through bone resorption. In boys, the

primary explanatory variables of radial SOS was NTX, followed by grip strength and maturity; where as in girls, it was maturity and dietary calcium. Longitudinally, maturity was found to have indirect effects on radial SOS mediated by grip strength. The influence of maturity on grip strength was similar between sexes, with the effect of grip strength on radial SOS being significantly greater in girls than boys (14.26 vs. 6.60; $p<0.05$); implying female bones maybe more responsive to muscle forces.

Together, these studies provide an overview of muscle-bone unit development during peri-pubertal maturation, demonstrating radial bone properties to be appropriately adapted to muscle function and force independent of physical activity.

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STATEMENT OF CONTRIBUTIONS

All of the work presented in this dissertation was conducted at Brock University and was approved by the University's Research Ethics Board [REB #05-155]. The research described herein was conducted at Brock University between 2010 and 2016. This work is to the best of my knowledge original, except where references are made to previous work.

For Chapters 1 and 2, I was responsible for chapter composition and involved in the formation of the theoretical framework for the two parts of this dissertation. A version of Chapter 3 has been published [Ludwa IA, Klentrou P. Physical activity interactions with bone accrual in children and adolescents. In: Y. Dionyssiotis (ed.), *Osteoporosis*. InTech Open Access Publishers, 2012]. I was responsible for reviewing all manuscripts using the eligibility criteria and search strategy outlined in this paper, as well as manuscript composition. I was also responsible for the composition of the updated systematic review.

A version of Chapter 4 has been submitted for publication and is currently under review [Ludwa IA, Falk B, Ward WE, Gammage KL, Klentrou P. Mechanical biochemical and dietary determinants of the functional model of bone development in children. *J. Musculoskeletal Neuronal Interactions*]. During the 4 years of conducting Part 2 of this work (Chapters 4 and 5), I was involved with all major areas of concept formation, data collection and analysis, and manuscript composition. Specifically, I was responsible in leading and attending all testing sessions with participants, where I conducted all of the anthropometric measurements, muscle size and grip strength assessments, all of the dietary interviews and analyses, all of the accelerometer data collection, its screening, and some of its raw data transformations, and all of the urine collection and analyses of NTX and creatinine. I also conducted one third of the radial SOS measurements. Lastly, I was responsible for the composition of Chapter 6.

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LIST OF ABBREVIATIONS

aBMD	Areal bone mineral density
BA	Bone area
BMAD	Bone mineral apparent density
BMC	Bone mineral content
BMD	Bone mineral density
BSAP	Bone-specific alkaline phosphatase
BSI	Bone strength index
BUA	Broadband ultrasound attenuation
BW	Body weight
CICP	C-terminal procollagen peptide
CSA	Cross-sectional area
DPA	Dual photon absorptiometry
DXA	Dual-energy X-ray absorptiometry
FM	Fat mass
FN	Femoral neck
HSA	Hip structural analysis
I^{mas}	Maximal moment of inertia
IGF-1	Insulin-like growth factor-1
LBM	Lean body mass
LS	Lumbar spine
MCSA	Muscle cross-sectional area
MVV	Moderate, vigorous and very vigorous

NN	Narrow neck
NS	Not significant
NTX	Cross-linked N-telopeptides of bone type I collagen
OC	Osteocalcin
PBM	Peak bone mass
PE	Physical education
PF	Proximal femur
PHV	Peak height velocity
pQCT	Peripheral quantitative computed tomography
PYD	Deoxypyridinoline
QCT	Quantitative computed tomography
QUS	Transaxial quantitative ultrasound
SM	Section modulus
SOS	Speed of sound
SSI	Stress-strain index
SXA	Single energy x-ray absorptiometry
TB	Total body
TR	Trochanter
TS	Tanner stage
vBMD	Volumetric bone mineral density
WBPA	Weight-bearing physical activity
WT	Wards triangle

CHAPTER 1

General Introduction

Optimizing skeletal development during growth and development is dependent on the extent of bone mineral that can be accrued during childhood and adolescence, along with the consolidation that continues beyond the attainment of final height (Davies et al., 2005). Studies in children and adolescents have shown increases in bone mass accumulation up to 18-20 years of age, with some acceleration during puberty (Bailey et al., 1996). Peak bone mass (PBM), defined as the highest level of bone mass achieved as a result of normal growth (Gordon, 2003), is largely achieved by age 18 to early 20s depending on the bone (Matkovic et al., 1994), with 90% of total body bone mineral content (BMC) being acquired by age 16 (Elgan et al., 2003). Recent literature suggests that the critical property of bone is strength, rather than mass (Schöenau & Fricke, 2008). Bone strength is important to preventing fractures, thus the aim of bone development should not be to increase bone mass but to make bones strong, with one of the means of achieving this goal being increases in bone mass (Schöenau & Fricke, 2008).

Moreover, bone accretion is a product of a complex interaction between genetic and environmental factors, including biochemical factors, diet, and mechanical stimuli (Klentrou, 2016). For example, in both boys and girls, estrogens have been shown to influence bone accrual, turnover, linear growth, apposition of bone on the endosteal surface, and epiphyseal closure, while androgens affect cortical bone size (Grumbach, 2000). Calcium and vitamin D

are also important factors to enhancing bone mineral acquisition in adolescents (Cadogan et al., 1997). Mechanical loading of sufficient intensity to promote an increase in skeletal mass requires the delivery of optimal strain to the bones on a regular basis. Some of the largest physiological loads placed on bone are the result of muscle contractions (Burr, 1997; Martin et al., 1998). Taking into account the balance between bone strength and the forces that normally challenge it, which in this case is muscle contractions, provides a functional approach to looking at how bone adapts to muscle forces. As such, the development of the muscle and skeletal system should not be considered separately but instead as a functional unit (Schönau et al., 1996), as a functional "muscle-bone unit", in which changes in muscle strength affect bone strength (Schöenau & Frost, 2002). This concept of a functional muscle-bone unit underscores that bone strength be related to muscle strength and function, and not necessarily to bone mass or age.

Mechanical loading and stimuli that influence bone development can come in the form of not only muscle contractions but also ground reaction forces. However, it is difficult to discern which of these forces cause the greatest adaptation to bone as they are not always mutually exclusive. One way to examine the effects of these forces separately is to investigate bone properties of the non-weight bearing versus weight bearing bones (Klentrou, 2016). Cross-sectional studies (Schöenau et al., 2000, 2002) have reported a positive association between surrogate measures of radial bone strength (cortical area or bone mineral content) and forearm muscle strength (muscle area) in boys and girls, with similar relationships observed for males and females before puberty. Likewise, weight-bearing activity was found to be the best contributor to lower limb bone strength properties (Duncan et al., 2002; Greene et al., 2005).

There are very limited data regarding longitudinal changes in the relationship between muscle and bone properties during growth in children. The majority of longitudinal studies have examined growth velocity of muscle size or strength in relation to the growth velocity of bone accretion at the radius or tibia (Rauch et al., 2004, Jackowski et al., 2009; Xu et al., 2009). Based on these growth velocities, the general consensus is that the changes in muscle size or strength precede changes in bone accretion or strength (Rauch et al., 2004, Jackowski et al., 2009; Xu et al., 2009); however, these results do not imply a cause and effect relationship. Only two studies (Wang et al., 2007; Wey et al., 2011) have examined the longitudinal relationship between muscle mass and strength on bone properties. The results of both these studies supported the previously described cross-sectional pubertal comparisons of Schöenau et al. (2000, 2002). In particular, Wey et al. (2011) found that controlling for physical activity level resulted in a tightening of the relationship between muscle and bone development, which highlights the importance of controlling for confounding variables when examining the functional muscle-bone unit during growth.

The rise in bone and muscular strength and size during youth is a reverse mirror image of the decline seen with aging. Thus, knowledge of the development of the muscle-bone unit and the assessment of its relationship will improve our understanding of the physiology and pathophysiology of bone development in children and adolescents, as well as with bone diseases such as osteoporosis that are closely associated with muscular function. Evaluating the functional muscle-bone unit may increase the sensitivity and specificity of fracture prediction in individuals if the focus shifts from bone mass to bone strength, and its relationship with the muscular system.

In order to explore and accurately describe the functional muscle-bone unit, relevant measures of both muscle strength and bone properties need to be measured (Klentrou, 2016). Thus, new longitudinal studies must go beyond consideration of the relationship between muscle and bone using growth velocity curves and instead examine changes in one tissue relative to the other. Finally, research examining the functional muscle-bone unit have focused on this relationship from a static perspective. Examination of biochemical markers of bone turnover, in addition to static measures of bone, is advantageous as it would provide a more complete understanding of the dynamic course of bone remodeling (Creighton et al., 2001; Szulc et al., 2000).

1.1 Thesis Approach

This dissertation involves a series of studies designed to assess changes in bone and muscle properties of healthy children over time. The overall purpose was to assess functional changes in the muscle-bone unit in order to determine if changes in muscle strength are directly related to changes in bone strength in addition to normal growth and maturation in peri-pubertal children, while also taking into consideration the influence of factors such as biological sex, age/maturity, bone metabolism, physical activity and nutrition.

The first part of this work was a systematic review of the literature on the effect of whole body physical activity on bone development in children. The purpose of this systematic review was to examine all available randomized control trials and controlled interventions geared at improving bone properties in children and adolescents in order to discern if there was a "type" of physical activity best suited to improving bone development and whether or not there was a specific "time" during growth to best introduce this activity. The review

implied that the best *time* to see largest improvements in bone properties may be during the peri-pubertal stages, however, the many different interventions employed did not make it clear what the best *type* of activity was, nor did they consistently separate the loading characteristics of muscle versus ground reaction forces.

Shown in Figure 1.1, the results of the review led us to the second part of this work, where we studied a low impact bone (the radius), from both a cross-sectional (study 1) and a longitudinal (study 2) perspective. The radius was chosen for two reasons: a) in order to separate the influence of muscle properties on bone strength from those of weight-bearing or ground reaction forces, and b) because the radius is a common fracture site in youth so these results may be clinically relevant for this population. Moreover, we used the functional model of bone development by Rauch and Schöenau (2001) to guide our examination. This functional model of bone development as proposed by Rauch and Schöenau (2001) is based on the Mechanostat Theory, according to which bones adapt their strength to keep the strains caused by physiological loads (i.e. muscle contractions) close to a set point (Frost, 1987; Rauch & Schöenau, 2001). Most importantly, this model takes into account the aforementioned non-mechanical factors (i.e. hormonal and nutritional) that influence bone metabolism and development and, in turn, regulate this set point (Figure 1.1).

PART 1

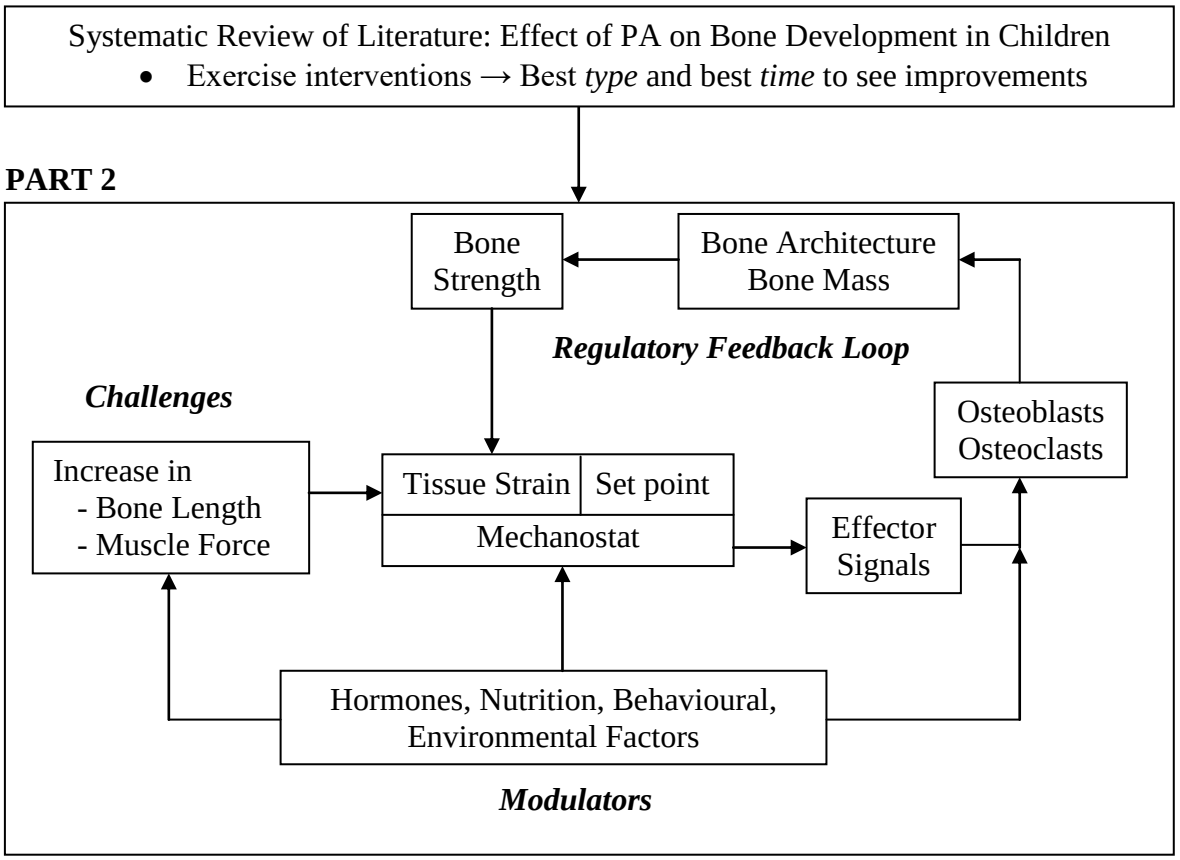

Figure 1.1 The Theoretical Framework centered around the Functional Model of Bone Development (from: the developing bone – slave or master of its cells and molecules? Rauch & Schöenau, 2001).

Specifically, the first study was a comprehensive cross-sectional evaluation of the functional model of bone development proposed by Rauch and Schöenau (2001) through examination of the relationship between muscle characteristics (size and strength) and non-weight bearing bone properties, as reflected by the speed of sound at the radius in peri-pubertal children. Behavioural factors (i.e., physical activity and nutrition) were also considered. For the first time, there was an attempt to include an indicator of bone resorption in the investigation of the muscle-bone unit. The second study involved a longitudinal examination of the normal changes in muscle and bone properties in children over 2 years,

using non-invasive measures and structural equation modelling. The specific objectives and hypotheses for both studies are listed in the next sub-section.

1.2 Objectives and Hypotheses

Part 1: The primary objective of the systematic review was to conduct a systematic review on the effectiveness of exercise and physical activity interventions to improve bone accrual and bone properties in children and adolescents. A secondary objective was to also discuss bone remodelling parameters influenced by exercise interventions. It was expected that such a review would help us to determine:

A) what is the best time during growth and development to influence bone health whether there is indeed a window of opportunity for bone response;

B) whether there is a modality that is best suited to improving bone development and to what degree such interventions influence changes in bone;

C) what are the characteristics of loading that have been shown to be best associated with particular structural improvements.

Part 2: The first observational study used a cross-sectional design to validate if previously observed relationships in the muscle-bone unit using BMC can also be demonstrated using non-invasive measures of bone strength, namely transaxial quantitative ultrasound (QUS), in order to support our next steps in the longitudinal examination of this relationship. Specific study objectives included:

A) Objective: Examine the relationship between dominant radial bone speed of sound (SOS) and muscle strength in peri-pubertal boys and girls.

Hypothesis: Radial SOS will be positively associated with forearm muscle strength (absolute and relative to muscle size) in both boys and girls. Specifically, grip strength will be a positive determinant of radial SOS independent of somatic maturity.

B) Objective: Determine which confounding variables influence the relationship between rSOS and forearm muscle strength in early pubertal boys and girls.

Hypothesis: Bone resorption, physical activity, and daily calcium intake, will act as significant correlates on radial SOS, with bone resorption and calcium intake, but not physical activity, acting as significant independent predictors of radial SOS.

The general purpose of the second observational study was to investigate if changes in bone properties at a non-weight bearing bone (i.e. radial SOS) are directly related to changes in forearm muscle strength in boys and girls across puberty. Specific study objectives included:

C) Objective: Examine changes in radial SOS in relation to changes in muscle strength (absolute grip strength) in boys and girls over a peri-pubertal period of 2 years.

Hypothesis: Radial SOS will increase with maturity in both boys and girls. Changes in grip strength will significantly predict radial bone SOS in boys and girls during their growth and maturation.

1.3 General Procedures of Part 2

The materials and procedures were identical for both studies; however, the variables analyzed, and the type of analyses conducted was different between studies. Measurements or indices of maturity, anthropometry, muscle size, muscle strength, bone strength, habitual

physical activity, nutrition and bone resorption were taken. This design allowed for the exanimation of the muscle-bone unit relationship from both a cross-sectional and longitudinal perspective.

1.3.1 Participants

In order to study the muscle-bone unit during growth, healthy (non-clinical or non-athletic) children and adolescents between the ages of 8-16 years were recruited and examined during a total of 4 years. Examination periods for the study occurred biannually, in the spring and autumn months, with each participant coming to the laboratory at Brock University annually during one of these testing periods (Spring 2010 - Spring 2013). During the first year of the study, 84 participants were recruited and tested. Of these 84 participants, 76 returned for a second year of assessment with an additional 74 new participants being recruited and examined. In the 3rd year of the study, 66 participants returned for a 3rd measurement occasion, 61 for a second measurement occasion, and 20 were newly examined participants. In the 4th year of the study, we conducted examinations during the spring session only and invited those participants for whom 2nd and 3rd year grip strength measurement occasions were missing in order to increase our sample size for longitudinal analysis. Participants who we suspected would be in the later stages of puberty (maturation) were also invited back to help increase the developmental range of participants for our cross-sectional analysis, as majority of the early recruited participants were either pre or early pubertal. During this final examination period, 44 participants returned for a 3rd measurement occasion and 8 for a 2nd occasion.

In total, 172 participants were examined during the course of study. Of these 172 participants, 41 participants had 3 measurement occasions, 96 had 2 measurement occasions and 35 had 1 measurement occasion. For the cross-sectional study, each participant was used only once in the analysis, and if there were multiple measurement occasions, the occasion for which there was the most complete data was utilized.

The same 172 participants from the cross-sectional analysis were used for the longitudinal study, along with all of their measurement time points, resulting in a total of 350 measurement occasions. Since all participants examined during the spring session of the first year (Spring 2010) had no grip strength measurement, the longitudinal analysis included the biannual sessions from autumn 2010 to spring 2013. After accounting for outliers and missing radial SOS and grip strength variables, a total of 290 measurement occasions were utilized in the actual longitudinal analysis. These 290 measurement occasions correspond to 129 participants involved in the first year measurement occasion, 115 in the second, and 46 in the third.

To avoid inter-observer variability, the author of this dissertation performed all the anthropometric and muscle size measurements, as well as the dietary recall interviews, consistently for the duration of the longitudinal study. Although every effort was made for one operator to solely perform all QUS measurements for the duration of the longitudinal study, this was not always possible. For the main operator the intra-operator coefficient of variation in 10 children was 2% and the interclass correlation coefficient (ICC) was 0.98. The inter-operator coefficient of variation was 3%.

1.3.2 Maturity

There are age- and maturity-dependent changes associated with the functional model of bone development during growth, and although not depicted (Figure 1.1), it is not always clear how and when to appropriately take into account the influence of these non-modifiable factors within the model. Age and maturity are often used as grouping or controlling variables in the examination of bone development in children, with the use of either variable leading to small differences in the amount of total variance explained in bone properties or potentially minor differences in explanatory variables of bone strength. During growth, the timing and tempo of maturation varies amongst children, and may not always coincide with chorological age. Furthermore, changes in bone and muscle properties do not tend to occur at the same chronological age, but occur approximately at the same maturational stage between boys and girls. Using maturity as opposed to age in our cross-sectional and longitudinal assessment of the muscle-bone unit allows us to mix our sample of boys and girls together as well as make meaningful comparisons between these groups based on their maturity level.

In part 2, somatic maturity offset (years from peak height velocity) was estimated using sex specific regression equations, based on known differential growth rates of height, sitting height and leg length, allowing for a single as opposed to serial measurement of these factors (Mirwald et al., 2002). Age of peak height velocity (PHV) is one of the most commonly used methods of assessing somatic maturity in adolescents (Malina et al., 2004; Mirwald et al., 2002). This method of maturity assessment has been used at an increasing rate in research (Barker et al. 2010; Falk et al. 2008; Ludwa et al., 2012; Macdonald et al. 2008; Moore et al. 2010; Nurmi-Lawton et al. 2004), although it has not yet been widely implemented in sports or clinical settings.

1.3.3 Transaxial Quantitative Ultrasound

One of the novelties of our design is the use of transaxial quantitative ultrasound (QUS). Both the cross-sectional and longitudinal studies investigating the muscle-bone unit to date have used dual-energy x-ray absorptiometry (DXA) technology to assess areal BMD, reflecting bone strength. In recent years, other methods available for estimation of bone density have been developed including quantitative computed tomography (QCT), and QUS.

The timing and magnitude of growth is a highly individualized and variable process, which has contributed to some of the equivocal results regarding the effects of body composition (lean versus fat mass) on bone in children and adolescents (Arabi et al., 2004; Cadogan et al., 1998; Goulding et al., 2000; Weiler et al., 2000). The variability in the results can be attributed in part to the various ways in which bone parameters are assessed. Bone strength is indirectly estimated by measuring the BMD using dual-energy X-ray absorptiometry (DXA), which is the major non-invasive measurement available for the early diagnosis of osteoporosis. This type of scanning has become the gold standard for diagnosing abnormalities in bone tissue in adults. Indeed, BMD is considered a major determinant of bone strength, and BMD values obtained at the level of the proximal femur and lumbar spine are used to diagnose osteoporosis after applying criteria established by a working group commissioned by the World Health Organization (Peck et al., 1993). Although DXA is the most commonly used and preferred method of bone mineral assessment, it is problematic in youth as it does not accurately account for differences in the size and shape of the bone (Bachrach, 2005). The major limitation of DXA is that it uses a 2-dimensional technique to quantify the 3-dimensional structure of bone.

Other methods available for estimation of bone density include quantitative computed tomography (QCT), and quantitative ultrasound (QUS). Peripheral QCT (pQCT) is a technique used mainly in research and has the ability to estimate true volumetric bone density by assessing 3-dimentional cross-sections of bone. By doing this, pQCT can offer valuable information on bone geometry, as well as BMC, BMD and even bone strength (Schoenau et al., 2004). Furthermore, pQCT has the ability to differentiate BMD and BMC between cortical and trabacular bone as well as provide information about the cross-sectional area of muscle. This is particularly advantageous when investigating the muscle-bone unit at the forearm, a common scanning location using pQCT. BMC of children and adolescents as assessed with QCT has been shown to be highly correlated ($r^2 = 0.94$) with the same measurements using DXA (Wren et al., 2005). The disadvantage of pQCT is its limited availability, and like DXA, is expensive and uses radiation (Specker & Schoenau, 2005).

Quantitative ultrasound has recently emerged as a promising technology for the assessment of bone strength. It is a particularly attractive technology because it is simple, relatively inexpensive, portable, non-invasive and radiation-free (Njeh et al., 1999; Schoenau et al., 2004). As a result, QUS has a greater potential for widespread application than fixed standard bone densitometry approaches, such as DXA and pQCT. Specifically, transaxial QUS is used for the assessment of skeletal properties, namely bone strength, by measuring speed of sound (SOS, m/s) along the bone. This is advantageous, because unlike DXA, QUS is not affected by bone size (Njeh et al., 1999) allowing for better comparisons between children of different sizes and ages (Baroncelli, 2008; Foldes et al., 1995). The SOS is measured using a hand-held probe, which contains a set of two transmitters and receivers that send sound waves along the length of the cortical bone. The use of this transmission method

along the bone does limit QUS to peripheral sites of the body such as the radius and tibia. The strength of bone is reflected by the time elapsed between the transmission and reception of the signal transmission, with faster transmissions reflecting stronger bone (Njeh et al., 1999). This measurement is based on the fact that the SOS will travel faster through bone than it will through soft tissue.

In vitro, QUS has been shown to assess previously unquantified properties of bone fragility (Gluer et al. 1993), with measures reflecting both quantitative and qualitative properties of bone such as BMD, elasticity and micro- architecture (Baroncelli, 2008; Jaworski et al., 1995; Prins et al., 1998). In particular, the transaxial transmission has been shown to be related to bone density and structure (Gluer et al., 1994) but not to cortical thickness (Njeh et al., 1999). It is important to mention that QUS is not a direct measure of bone strength but the SOS score reflects bone strength as it takes into account both the qualitative and quantitative aspects that make up bone strength (Foldes et al., 1995). Although it does not determine the percent contribution of these aspects separately, it does allow for the simultaneous measurement of these factors by providing a composite score.

QUS fits World Health Organization criteria for osteoporosis diagnosis. In part 2, bone SOS was measured using the QUS, Sunlight Omnisense™ System (Sunlight Medical, Israel). The Sunlight Omnisense^{TM} System has been used to assess bone properties at various sites such as the radius, ulna, metacarpal and phalanx (Barkmann et al., 2000; Hans et al., 2003), to discriminate between women who have had a fracture and healthy, age-matched women who have not had a fracture. Previous studies have demonstrated that QUS can predict fracture risk independent of BMD in the elderly (Bouxsein et al., 1999; Gonnelli et al., 2005), as well as predict site-specific (vertebral and hip) fractures (Bauer et al.1995; Njeh et al. 1997).

Furthermore, QUS assessment has demonstrated lower SOS values in children with fracture compared to healthy controls (Schalamon et al., 2004), and has been suggested as a useful method to assess bone quality and fracture risk in children and adolescents with bone and mineral disorders (Baroncelli et al., 2003). It has also been used, although to a limited extent, to demonstrate the effect of growth, body composition, dietary intake, and exercise training or physical activity on various bones (e.g. calcaneous, tibia, radius) and in different age groups (Daly et al., 1997; Eliakim et al., 2001; Falk et al., 2000, 2003, 2008; Litmanovitz et al., 2003; Ludwa et al., 2010; Yao et al., 2011). On the other hand, QUS has not been used in other longitudinal studies because unlike DXA the clinical usefulness of QUS has yet to be investigated, and comparison studies have shown inconsistent correlations with DXA (Baroncelli, 2008; Sioen et al., 2011). Furthermore, QUS is limited to peripheral sites, and does not have the ability to differentiate between various skeletal properties like pQCT. When evaluating bone strength and its properties in the pediatric population it is important to consider changes experienced during periods of rapid growth during puberty. There will be rapid changes in both the size and shape of bone, and in turn how these parameters are measured using the aforementioned bone assessment techniques. This can be problematic when attempting to investigate a large number of children due to the great variability in bone development during this time. Using QUS as a reflection of bone strength may be advantageous, as it is not affected by changes in bone size and allows for better comparisons in growing youth. Moreover, since QUS reflects bone strength of cortical bone at the radius, it allows us to meaningfully discuss our study results with the known sex-specific changes in cortical development in the shafts of long bone during growth (section 2.3).

Although these imaging techniques have been used in the majority of studies examining the relative importance of mechanical and dietary factors on bone, they only provide a static rather than dynamic picture of bone. Examination of biochemical markers of bone turnover, in addition to static measures of bone, can aid in the study of skeletal metabolism and growth by providing an understanding of the dynamic course of bone remodeling (Elgan et al., 2003; Fares et al., 2003). Limited research exists examining musculoskeletal interactions from a metabolic perspective, let alone in combination with methods other than DXA, while considering factors of physical activity and nutrition, and in children.

1.3.4 Bone Resorption

Ninety percent of bone matrix is comprised of a structural protein called collagen. The majority of this collagen is type-1 collagen that is cross-linked by either pyridinolines (Pyr) or deoxypyridinolines (Dypr) (Mora et al., 1998), with cross-links also occurring at both the amino- and carboxy-terminal ends of the teleopeptide (N-and C- terminal telopeptides, NTX and CTX, respectively) (Szulc et al., 2000). The cross-linked collagen infrastructure of bone undergoes continuous remodelling that involves osteoclast mediated resorption resulting in the production of these cross-linked structures. Assays have been developed to ascertain the amount of osteoclast activity occurring and determine the degree of bone resorption by measuring these markers in blood and urine (Eastell et al., 2000; Seibel, 2002; Szulc et al, 2000; Watts, 1999).

For the purpose of this dissertation, bone resorption was determined by measuring the resting levels of cross-linked N-teleopeptide of type I collagen (NTX) in morning urine

(expressed in nM BCE/mM creatinine). Markers of bone resorption are excreted in blood and urine following the breakdown of collagen during bone resorption. NTX has been established as one of the newer markers of bone resorption specific to the metabolic break down of bone collagen, as those cross-links formed at the amino-terminal portion of the type-1 collagen seem to be generated only by osteoclasts making NTX a specific indicator of bone resorption (Eastell et al., 2000; Mora et al., 1998). Urinary NTX levels have been shown to be higher in both male and female children compared to adults (Sato et al., 2010), with concentrations decreasing to adults' levels following the adolescent growth spurt (Mora et al., 1998; Szulc et al., 2000). Moreover, NTX is higher in girls than boys early in adolescence with higher values being observed in boys compared to girls later on in adolescence (Mora et al., 1998). NTX concentrations have also been observed to be highest in the morning and lowest in late evening (Szulc et al., 2000), with first morning voids having higher levels of NTX compared to second morning voids (Sato et al., 2010). Furthermore, urine levels of NTX have been established as a specific indicator of the current level of bone resorption (Eastell et al., 2000, Sato et al., 2010).

The International Osteoporosis Foundation and the International Federation of Clinical Chemistry recommends the use of serum type 1 procollagen N-terminal propeptide (P1NP) and CTX as markers for formation and resorption, respectively. This decision was based on the following criteria: adequate characterization of the marker, their specificity to bone and performance in clinical studies, availability, biological and analytical variability, sample handling, stability, ease of the analysis, availability of method in routine laboratories, potential for standardization of methods and the medium of measurement (serum vs. urine). Due to the difficulty in obtaining serial serum samples in children and the potential fear of having blood

drawn, we instead opted for urine collection to ensure a sample was provided from each participant at each measurement occasion as well as helping to limit any participant drop outs due to serum collection. In a clinical setting, urine NTX has been suggested to be the preferred marker over plasma CTX as it is not as sensitive circadian changes and is not affected by food intake (Wheater et al., 2013). As in our case, urine collection also avoids the invasive venepuncture procedure associated with blood sampling and is often preferred by patients (Baxter et al., 2013; Wheater et al., 2013). For these reasons, we decided to estimate a marker of bone resorption using urinary NTX.

1.3.5 Muscle Size and Strength

Muscle size and strength increase during growth and maturation. Moreover, there are age- and sex-associated changes in muscle strength during childhood. Grip strength has been extensively investigated throughout the literature in children. The age-related changes in grip strength are similar between boys and girls until the onset of puberty, with grip strength advancing almost linearly from early childhood until puberty (Blimkie, 1989; Malina et al., 2004). During the pubertal period, the rate and size of strength increases begins to vary between sexes. In boys, there is a rapid and considerable increase grip strength before eventually slowing down during adolescence (Blimkie, 1989). Although grip strength appears to continue to increase in girls during puberty, the rate of increase does not increase as substantially compared to boys and the rate of increase is similar to that observed in the prepubertal period (Blimkie, 1989). In fact, there appears to be very little increase in grip strength in girls during adolescence. Therefore, sex-related differences in grip strength are rather small before the male adolescent growth spurt, after which the differences in strength

become progressively larger with increasing age (Blimkie, 1989). Since girls mature earlier than boys, they achieve 50% of their peak final grip strength by the age of 10 years, compared to 12.5 years in boys (Blimkie, 1989).

Muscle strength is largely a function of muscle size, with the significant gains in muscle strength occurring during puberty being largely attributed to increases in muscle mass (Sale & Spriet, 1996). As a result, muscle size or mass are strong correlates of muscle strength and are often used as surrogate measures for muscle strength when strength assessments are not able to be made. Even when muscle strength is normalized according to body size, mass, or specifically muscle size, the age-related differences in grip strength between sexes persists (Blimkie, 1989; Malina et al., 2004).

In Part 2, isometric grip strength was used to assess forearm muscle strength. Isometric grip was chosen due to it being widely studied and reported within the literature for both sexes throughout childhood and adolescence (Blimkie, 1989). Moreover, grip strength is a simple, quick and inexpensive method that can reliably measure strength in growing children. Using grip strength to help determine the influence of maturation on the functional muscle-bone unit is advantageous due to its known sex- and age-related changes that reflect not only changes in muscle mass and strength, but similar patterns of change in bone mass and strength, during growth. More importantly, grip strength was selected due to its close proximity to the distal radius, at which we were attempting to estimate bone strength. This close proximity allows for a better examination of the functional relationship of the muscle-bone unit than using surrogate measures of strength, such as muscle cross-sectional area (MCSA) or global measures of lean body mass (LBM). Though not often used concurrently with assessments of bone strength, grip strength has been used to assess muscle strength in the muscle-bone unit in

children (Gracia-Marco et al., 2011; Herrmann et al., 2015; Vicente-Rodríguez et al., 2008), clinical populations (Okumus, et al., 2006; Tenbrock et al., 2000), and adults (Frank et al., 2010; Hasewega et al., 2001; Lorbergs et al., 2011).

1.3.6 Physical Activity and Nutrition

Accelerometers measure physical activity by quantifying both the volume and intensity of movement (Freedson et al., 2005) by measuring vertical acceleration. The displacement of the body is analyzed by an internal cantilever beam within the accelerometer, which emits a charge proportional to the acceleration of the body, or the limb, and digitizes the movement into "counts" (vertical accelerations). The counts are then summed over a specified duration or epoch which can be used to determine the time spent in light, moderate, hard and very hard physical activity based on age- appropriate cut offs (Freedson et al., 1997; Trost et al., 2001). Moreover, as these counts represent vertical accelerations of the body they can be related to ground reaction forces, which is important when looking at weight-bearing bone development (Janz et al., 2003). Since we are attempting to minimize the impact of weight bearing physical activity (WBPA) on radial SOS scores, using accelerometry to measure activity is advantageous in helping us to distinguish the loading effects from muscle strength on bone.

Previous research has demonstrated Actigraph accelerometers to have excellent intra- and inter-instrument reliability across a wide range of accelerations (Eslinger & Tremblay, 2006). Furthermore, results of physical activity assessed using these accelerometers have been shown to be correlated (r=0.53-0.73) with children's free play activities assessed with heart rate monitors and direct observations (Ott et al., 2000), and whole-room calorimetry (Puyau et al., 2002).

Dietary intake was evaluated using a 24-hour recall interview, as previously described (Moore et al., 2007), which was also performed consistently by the author for the duration of the longitudinal study. The 24-hour recall method is the most commonly used assessment tool in large cross-sectional surveys and skeletal development studies in both children and adults (Moore et al., 2007). This method provides a valid estimate of energy intake and calcium intake in adolescent females (Greger & Etnyer, 1978). This method also has numerous advantages including responsiveness to change in food supply and habit (Guenther et al., 1997; Harrison et al., 2000). Using a 24-hour recall interview was advantageous in getting a detailed report of nutritional intake in children and adolescents and was particularly helpful in the younger aged participants who would have likely had difficulty filling out a take home food record or questionnaire. It also allowed for the interviewer to guide the participants' recall with the help of a parent to gather the most accurate information about food preparation and amount of intake. Using pictures to represent different portions sizes and prompt accurate recall was that much more important in ensuring that intake was not over or under reported.

CHAPTER 2

Bone Development and Adaptation – an Overview of the Literature

2.1 Introduction

Osteoporosis is a condition characterized by low bone mass and reduced bone strength due to deteriorating bone tissue, resulting in bone fragility and propensity to fracture (Gordon, 2003; Munch & Shapiro, 2006; Peck et al., 1993). It is associated with high morbidity and increased mortality, and is considered worldwide to be a major health problem. According to the World Health Organization, osteoporosis affects approximately 200 million women worldwide (Kanis, 2007), with 1 in 3 women and 1 in 5 men suffering from an osteoporotic fracture during their lifetimes (Osteoporosis Canada, 2012). Although the prevalence of fractures is higher in women, the mortality rate related to fragility fractures is higher in men (Center et al., 1999; Hasserius et al., 2003). Moreover, bone fracture is not only due to decreased bone mass or alteration of the microarchitecture of bone, but is also related to falls as a result of loss of balance, inappropriate protective responses, or muscle weakness (Ammann et al., 1998; Bonjour et al., 1999). Although osteoporosis is considered a disease of the elderly, factors affecting bone strength are most influential during growth and development in youth.

The strength of our bones is influenced by both intrinsic and extrinsic factors. Intrinsic factors, those over which we have no control, influence our bone strength to a larger degree than extrinsic factors (approximately 80%) and can include genetics, family history, ethnicity, sex, and hormonal milieu (Gordon, 2003; Steelman & Zeitler, 2001). On the other hand, extrinsic factors are under our volitional control, with behavioural factors such as nutrition and physical activity contributing up to 20% of our total bone strength (Gordon, 2003; Steelman & Zeitler, 2001). As such, the development of lifelong strong and healthy bones is a complex interaction between these genetic and environmental/behavioural factors. Therefore, the aim of this review is to describe the properties that contribute to bone strength and the theoretical mechanisms used to explain how bones adapt to various external loads during childhood and adolescence. These external loads will be examined from the perspective of physical activity and mainly, muscle contractions. Furthermore, the effects of these internal and external factors and their influence on bone and muscle, separately and together, will be discussed.

2.2 Bone Morphology

Bone is a living tissue that makes up the body's skeleton. There are 206 bones in the human skeleton, not including teeth and sesamoid bones (small bones found within cartilage) that are classified by their shape as long, short, flat, and irregular; primarily, however, they are referred to as long or short. These bones are divided into the axial (central anchor) and appendicular skeletons. The 80 axial bones of the head, facial, hyoid, auditory, trunk, ribs, and sternum have a thin cortical shell and rich cancellous network and are located adjacent to the viscera (Marieb & Hoehn, 2016). The 126 appendicular bones in the upper and lower

extremities have thick cortical shells with cancellous bone in the epiphyseal regions and metaphyseal regions, and are surrounded by muscles (Marieb & Hoehn, 2016).

Bone (osseous) tissue is considered a connective tissue consisting of both cells and the materials such as collagen that these cells secrete (Gupta & Zioupos, 2008; Marieb and Hoehn, 2016; Rho et al., 1998). The bone tissue also serves as a reservoir for minerals, including calcium and phosphate, in the form of crystals, known as hydroxyapatite (Weiner & Traub, 1992). The bone marrow fills the porous central cavities of the diaphyses (shafts) of bones, and is the site for blood cell production (Marieb & Hoehn, 2016). Bone tissue contains hematopoietic cells, which can produce blood cells and stromal cells, which can produce fat, cartilage and bone.

There are two types of bone compartments: cortical bone and trabecular bone. Cortical bone constitutes about 80% of adult skeletal mass (Gupta & Zioupos, 2008; Marieb & Hoehn, 2016) and is dense with well-defined periosteal and endosteal surfaces (Gupta & Zioupos, 2008; Marieb & Hoehn, 2016). Cortical bone is heavily mineralized to provide structural support and is located in the diaphysis of the long bones whereas trabecular bone is calcified to a lesser extent than cortical bone and found at the ends of bone (Gupta & Zioupos, 2008; Rho et al., 1998). As a result of the low surface to volume ratio and small surface adjacent to the marrow, there is a low turnover rate in cortical bone (Gupta & Zioupos, 2008). Trabecular bone has a greater surface area than cortical bone, which allows it to be more metabolically active (Gupta & Zioupos, 2008; Marieb & Hoehn, 2016; Rho et al., 1998). Greater metabolic activity allows the trabecular bone to be more responsive to hormonal factors. On the other hand, as will be discussed in the next sections, cortical bone constantly remodels itself in

response to changing mechanical and non-mechanical environmental signals and microdamage (Gupta & Zioupos, 2008; Kjaer et al., 2015).

2.3 Bone Development

Bone growth during childhood and adolescence is of major importance for the size of peak bone mass. During puberty there is a considerable increase in bone mass resulting in an increase in bone size (length and width) and bone density (Molgaard et al., 1999). BMC gains during adolescence are more a function of somatic maturity than chronological age (Bailey et al., 1996), with an observed dissociation between statural growth and gains in total body bone mass as well as bone area (Faulkner et al., 2006). Peak statural growth, peak height velocity (PHV), occurs earlier in puberty compared to maximal bone mineral accumulation. In fact, the age of peak linear growth occurs approximately 0.5 and 1 year prior to peak gains in bone area (Faulkner et al., 2006) and BMC (Bailey et al., 1996), respectively, and 2 years before menarche in girls (Cadogan et al., 1998). With peak gains in bone accrual occurring after bone growth in length and width (Bailey et al., 1996; Molgaard et al., 1999), the density of bone may be lower than optimal due to changes in bone size occurring before its mineralization. As a result, linear bone growth may not have sufficient periosteal apposition and may leave bone more susceptible to fracture.

Specifically, in females, the time of maximum bone acquisition occurs between 11–14 years of age, a time that corresponds to pubertal stages Tanner 3–5 (Bonjour et al., 1991; Theintz et al., 1992). The age at which peak bone mass occurs varies depending on the bone. The lumbar spine (L2-L4) and femoral neck reached peak bone mass between the ages of 14-15 years, before the midfemoral shaft at 17-18 years of age (Bonjour et al., 1991).

Approximately 50% of peak bone mass is accrued around the time of PHV in girls (Cadogan et al., 1998), with 90% of total body BMC accrued by the end of the second decade (Elgan et al., 2003; Stager et al., 2006), and the remaining 5-10% achieved by the third decade (Cadogan et al., 1998). In boys, lumbar spine bone mass more than doubles between the ages of 11-17 years (Bonjour et al., 1991). During growth and maturation, girls experience their growth spurt earlier than boys, and early in adolescence, resulting in greater total BMC during this time (Faulkner et al., 1996; Malina et al., 2004). Conversely, by late adolescence, total BMC is greater in boys. This difference is due to BMC accrual plateauing in girls around 15-16 years of age, while BMC continues to increase in boys into their 20's (Faulkner et al., 1996). This pattern is also reflected in the development of BMD and translates to boys having greater BMC later on in life compared to girls (Faulkner et al., 1996).

There are also sex differences in the development of cortical bone and the sites where bone is deposited during puberty. Before puberty, both sexes undergo periosteal expansion and endocortical resorption (Garn, 1972; Kontulainen et al., 2006). During puberty, boys mainly add bone on the periosteal (outer) surface of the bone, whereas girls add bone to the endocortical (inner) surface (Shoenau et al., 2001). This is reflected in greater gains in periosteal diameter in boys and a narrowing of the endocortical diameter in girls, resulting in greater overall bone size seen in boys (Garn, 1970; Seeman, 1997). Furthermore, the addition of bone to the endocortical surface does not contribute much to bone strength, unlike the addition to the periosteal surface, which greatly increases the integrity of bone (Shoenau et al., 2001). The increased apposition of bone on the endocortical surface in girls is believed to act as a calcium reservoir for future reproduction and lactation (Garn, 1972; Kontulainen et al., 2006; Shoenau et al., 2001). Furthermore, these sex differences in the deposition of bone

continue into later life. With age, bone is lost primarily from the endocortical and intracortical surfaces making cortical bone thinner and more porous (Garn, 1970; Seeman, 1997). In order to increase the diameter of bone and maintain its strength, bone may be added to the periosteum (Beck at al., 2001; Seeman, 2002; Seeman & Delmas, 2006). This process appears to be more efficient in men compared to women and may explain some of the observed differences in fracture rates between sexes.

Maximizing peak bone mass (PBM) is advocated as the best way to prevent osteoporosis as it is generally accepted that those who achieve a higher PBM are at less risk of experiencing an osteoporotic fracture later in life (Bonjour et al., 1991; Molgaard et al., 1999). PBM defined as the highest level of bone mass achieved as a result of normal growth (Gordon, 2003). However, recent literature suggests that the critical property of bone is strength, rather than mass (Schöenau & Fricke, 2008). Bone strength is important to preventing fractures, thus the aim of bone development should not be to increase bone mass and make them heavier, but to make bones strong, which is only partially achieved by increasing bone mass (Schöenau & Fricke, 2008).

Therefore, puberty is a time of large increases in bone mass over a relatively brief period. The bone mineral accumulation that continues after the cessation of longitudinal growth allows for other factors to influence the accrual of bone and in turn its strength. In fact, any condition that impairs this process may create a deficit in bone mass with associated permanent ramifications for future bone strength. Careful evaluation of the factors associated with the increase in bone mass during this phase may be important for prevention of osteoporosis later in life, as the amount of bone accrued during growth may be a major determinant of future susceptibility to fractures.

2.4 Bone Strength

Bone fractures occur when the load applied exceeds the strength of bone. The skeletal characteristics that contribute to bone strength include the *quantity* and *quality* of bone. The *quantity* of bone material is the *mass* component of bone and is represented most often by bone mineral density or content (BMD and BMC, respectively). BMD and BMC are thought to be major determinants of bone strength because they can account for up to 60-70% of the variability seen in its strength (Ammann & Rizzoli, 2003). Dimensions of the bones, such as external diameters and cortical thickness, are major determinants of bone strength. The outer diameter of the long bones predicts up to 55% of the variation in bone strength (Ammann et al., 1996; 1998).

The factors that contribute to the *quality* of bone strength refer to the condition of the material and to how it is distributed via the structure or geometry of bone (Klentrou, 2016). The actual quality of material refers to the mineralization, elasticity, fatigue damage and even the porosity of the bone (Ammann & Rizzoli, 2003; Turner & Robling, 2004). The parameters of bone geometry, bone volume, cross-sectional area and cortical thickness have been shown to be positively related to each other as well as bone strength, and like BMD and BMC, can also account for up to 80% of the variance in bone strength (Voide et al., 2008). Since DXA-measured BMD only accounts for 60–70% of the variation in bone strength (Klentrou, 2016), some important factors are not captured by DXA in the progression of osteoporosis and the effects of anti-osteoporotic treatment. Geometry and trabecular microarchitecture must also be taken into account (Klentrou, 2016). Thus, the assessment of intrinsic mechanical quality of bony tissue should provide a better understanding of the role of tissue quality in determining bone strength (Amman & Rizzoli, 2003). In general, BMD remains a good predictor of bone

strength but it is only a surrogate determinant of bone strength. However, although BMD and BMC are predominantly used as surrogates of bone strength it is important to remember they are not the only factors contributing to the integrity of bone. There is a large body of evidence that suggest that bone strength is determined by various parameters such as bone geometry, cortical thickness and porosity, trabecular bone morphology (Amman & Rizzoli, 2003), and intrinsic properties of bony tissue (Klentrou, 2016).

The problem is that BMD and BMC are not mechanical properties and it can be argued that structure is more important and plays a larger role in bone strength. In older women, more than 50% of fractures occur in those with normal BMD, who are not diagnosed as osteoporotic based on current criteria (Stone et al., 2003; Wainwright et al., 2005). As previously mentioned, the surface on which bone is deposited during growth can affect its strength. Consider the shafts of long bones as cylindrical tubes. Adding mass to the outside surface of these bones makes them stronger, having a greater bending strength (section modulus), because the mass is further away from the neutral axis (Petit et al., 2005; Snow-Harter & Marcus, 1991). The expanding periosteal diameter will increase bone bending strength despite reductions in areal or volumetric BMD, meaning less material is needed for similar bending stiffness (Petit et al., 2005). These structural effects are important in adults, but critical in children as their bones are changing in both length and width during growth. The previously described sex-related differences in bone deposition (periosteal versus endosteal in boys and girls, respectively), may also account for the differences in bone strength observed between sexes during growth and maturation. The changes in limb length, and in turn the lever arms, of long bones are driven by muscle actions making bending forces the dominant load experienced and the distribution of bone to the surface more important than

the amount of bone added (Petit et al., 2005). Therefore, it can be argued that bone structure, its *quality*, may be more important to bone strength than the *quantity* of bone.

2.4.1 Biological Properties

The dynamic cellular activities of bone growth, modeling and remodeling are regulated by the bone's hormonal and mechanical environment through three types of bone cells: bone resorbing (osteoclasts), bone forming (osteoblasts) cells, and osteocytes that can sense strain or stress placed on the bones, although probably this is not their only function (Parfitt, 2002). Longitudinal bone growth occurs by a process called endochondral bone formation and involves two steps: (1) cartilage is added to the growth plates located at both the proximal and distal ends of long bones; and (2) cartilaginous scaffolding is replaced by bone tissue in the adjacent metaphyses (Bayliss et al., 2012).

Bone modeling and remodelling occur through the independent action of bone deposition by osteoblasts and resorption by osteoclasts. Osteoblasts are a general class of mesenchymal cells that form bone by synthesizing collagen matrix and then secreting calcium-phosphate mineral (Pearson & Lieberman, 2004). Typically, osteoblasts deposit fresh bone matrix to the periosteal surface of bone, while osteoclasts on the endocortical surface resorb it (Schöenau et al., 2004). Modeling affects the size and shape of long bones by increasing the outer circumference of bone as well as the size of the marrow cavity (Parfitt, 2002; Schöenau et al., 2004). When the rate of periosteal apposition exceeds that of endocortical resorption, the cortex widens and shifts further away from the central long axis of the bone, strengthening the bone (Seeman, 2008). Therefore, bone modeling leads to a net gain of bone over time through the independent action of these cells and is important to

reshaping long bones during growth as well as to regional responses to mechanical loading conditions (Bailey et al., 1996; Parfitt, 2002).

Bone remodeling refers to the *coupled* action of osteoblasts and osteoclasts on the same bone surface, thus preserving the acquired bone mass (Bailey et al., 1996; Parfitt, 2002; Schöenau et al., 2004). Remodeling occurs by successive cycles of bone resorption and formation by first resorbing a pit of old bone and subsequently mineralizing new bone at the same location. The principal purpose of this process is to replace fatigue-damaged bone incurred by repetitive loading and results in the continuous turnover of bone (Bailey et al., 1996; Parfitt, 2002; Schöenau et al., 2004). This process happens throughout the lifespan and is widespread within the skeleton; however, with aging, remodeling results in a net loss of bone particularly on the endosteal surface (Schöenau et al., 2004), which leads to weaker bones and susceptibility of fracture. Clinical studies indicate that markers of bone remodeling could be independent predictors of the risk of fracture (Garnero et al., 1996).

2.4.2 Biomechanical Properties

Mechanical forces have a major influence on bone (re)modeling processes which help bone adapt to be able to withstand fracture from external loads by making them stronger. When bones are loaded, they experience some type of stress through compression or torsion, and, depending on the magnitude of force applied, some form of strain as well (Einhorn, 1992; Pearson & Lieberman, 2004). Stress refers to the internal resistance of bone and is equal in magnitude but in opposite direction to an applied force. Strain, on the other hand, describes changes in dimension and the amount of deformation that the bone tissue experiences from an applied external force (Einhorn, 1992). Therefore, bone strength and its resistance to fracture

is reflected in this stress-strain association, which actually is a load versus deformation relationship.

The amount of stress that is applied to bone will dictate its reaction to the stimulus. At low to moderate levels of stress, there is a linear relationship between the applied load and the resulting deformation. This linear relationship between stress and strain is known as Young's modulus and represents the elastic region of bone that is considered a measure of stiffness or rigidity (Einhorn, 1992; Pearson & Lieberman, 2004). Loading in this region allows for bone to behave like an elastic, meaning that the forces applied will deform the bone temporarily and allow for the bone to return to its original shape once the stimulus is removed. Deformation only becomes permanent when the applied force moves the bone beyond its elastic limit (into the plastic region) at which point the stress-strain relationship is no longer linear (Einhorn, 1992; Pearson & Lieberman, 2004). The upper limit of the plastic region is known as the point of failure and represents the maximum stress and strain bone can withstand before it fails. Any stress beyond this point will result in fracture (Einhorn, 1992).

According to Martin and Burr (1989), when strain is applied to bone, an osteogenic (i.e., bone formation) response will be initiated through the activation of osteogenic cells (i.e., osteoblasts) and lead to one of four potential outcomes. The first outcome is no response (quiescence), either because the signal was not sufficient (e.g., below a threshold value) or because the response was inhibited. The second outcome involves osteoblasts being recruited in the periosteum or endosteum to grow new bone (i.e., modeling). The third outcome is resorption, in which osteoclasts are recruited to resorb bone along a surface (i.e., resorptive modeling). The fourth outcome is bone turnover, also known as Haversian remodeling in

cortical bone. This process occurs through a coordinated, sequential activation of a bone metabolic unit involving osteoclasts and osteoblasts together (Martin & Burr, 1989).

What is of interest is how these biological and biomechanical properties combine to contribute to bone strength. The dynamic cellular activities of bone (re)modeling change the quantity and quality of bone, which makes them stronger. In this case, the stress-strain relationship can still be used to parallel the reaction of bone to external forces, but what changes is how the stronger bone responds to newly applied loads. A stronger bone will shift the biomechanical regions and limits within the stress-strain relationship, which would require a greater magnitude of force to deform and ultimately fracture the bone. The stress-strain relationship can therefore be used to describe bone strength and its response to externally applied forces by providing a framework for how the qualitative and quantitative skeletal characteristics previously discussed can contribute to the biomechanical properties of bone. However, the stress-strain relationship fails to elucidate the *process* by which bones *adapt* to external loads.

As previously mentioned, when bones are loaded in compression, tension, or torsion, bone tissue is deformed causing it to be strained. The strain on bone tissue causes fluid within the bone to move past the cell membrane of osteocytes (Bonewald, 2006). Osteocytes are mature bone cells embedded throughout bone matrix that are connected with one another, with other bone cells (osteoblasts and osteoclasts), and with the bone marrow through dendritic processes (Bloomfield, 2001; Sims & Gooi, 2008). Recent literature has shown that osteocytes can act as mechanosensory cells and that fluid flow along the osteocyte causes a release of molecular signals initiating recruitment of osteoclasts and osteoblasts to (re)model bone in response to the mechanical load provided (Bloomfield, 2001; Bonewald, 2006; Datta et al.,

2008). The *process* of turning a mechanical signal into a biochemical signal is called mechanotransduction.

2.5 Bone Adaptation

The understanding that bone can adapt to its mechanical environment has been speculated for some time. This concept originated from 19th century ideas that bones respond to interactions with their mechanical environment by altering their architecture through remodeling (Frost, 1994, 1998; Pearson & Lieberman, 2004). The proposition that bones are remodeled throughout life to adapt to external loads, potentially leading to a predictable relationship between structure and function (a functional adaptation), is generally known as Wolff's Law. According to this law, every change in the form and function of bone or of its function alone is followed by certain definite changes in its internal architecture, and equally definite alteration in its external conformation (Frost, 1994, 1998).

Wolff observed that the orientation of bone, particularly the trabeculae, corresponded to the direction of applied loads. This observation lead him to propose that bone loading could somehow' be sensed, which allows for bone to adapt accordingly (Pearson & Lieberman, 2004). Wolff's Law marked a step forward in the understanding that mechanical influences can change bone architecture, but states neither *how* bone adapts to its environment nor that adaptation involves a change in bone strength.

2.5.1Mechanostat Theory

It had been recognized that bones are responsive to mechanical loading. However, Harold Frost was the first to provide a detailed theory regarding *how* load-bearing bones adapt

to maintain their integrity in response to applied external loads. Frost suggested the response of bone to its mechanical environment is controlled by a "mechanostat", its own homeostatic control system, which aims to keep the bone tissue stress-strain relationship at an optimal level (Frost, 1987, 2003). This homeostatic regulatory mechanism senses changes in the mechanical demands placed on bone and subsequently alters its mass and architecture in response to these new demands, in turn altering bone strength and potentially its mechanical set-point (Frost, 1987, 2003). Thus, bone tissue has an intrinsic "mechanostat" regulating bone adaptation.

As with any homeostatic control system, bone's mechanostat must include several independent components such as a stimulus, a sensory and effector process that is capable of detecting the stimulus and translating it in order to keep bone deformation within a set-point. The stimulus for bones to adapt their strength comes from the amount of strain placed on the bone (Martin & Burr, 1989). This stimulus will signal the dynamic cellular activities of osteoblasts and osteoclasts to become the effector mechanism (re)modeling bone and its strength (Martin & Burr, 1989; Frost, 2003). Frost postulated several mechanical thresholds which would control this (re)modeling process and whether bone is added or taken away from the skeleton. Mechanical use below a certain threshold would result in bone being resorbed and a loss in bone mass, while mechanical use above a particular threshold would cause bone formation and changes in structure to increase bone strength (Frost, 1987; Martin & Burr, 1989).

Frost's mechanostat does not fully explain the cellular level mechanisms behind this (re)modeling process, particularly how the mechanical stimulus is detected and sensed to how it is translated to cause bone formation and resorption by the aforementioned effector cells. As

previously mentioned, it appears that osteocytes may play an important role as the sensory cells in this cellular mechanism, as it has been revealed that these cells have the ability to sense and respond to mechanical stimulation through mechanotransduction (Bloomfield, 2001; Bonewald, 2006; Datta et al., 2008). Therefore, the action of these various components would act as a regulatory feedback loop allowing for bone to adapt and adjust its strength to changing external stimuli (Figure 2.1).

2.5.2 Functional Model of Bone Development

The functional model of bone development as proposed by Rauch and Schöenau (2001) has Frost's Mechanostat Theory at its core. The functional model of bone development postulates that the primary mechanical challenges to bone's *mechanostat* during growth comes from increases in bone length and muscle force (Frost, 1987; Rauch & Schöenau, 2001). This suggests that the growth of bone and muscle are closely associated, and that bone must adapt its strength to withstand forces from muscle contractions (Schöenau and Fricke, 2008; Schöenau & Frost, 2002). Bone development is a product of a complex interaction between genetic and environmental factors including nutritional and hormonal influences, as well as mechanical stimuli (Gordon, 2003; Steelman & Zeitler, 2001). Most importantly, this model takes into account the aforementioned non-mechanical factors (i.e. hormonal and nutritional) that influence bone metabolism and development and, in turn, regulate bones mechanostatic set point (Figure 2.1).

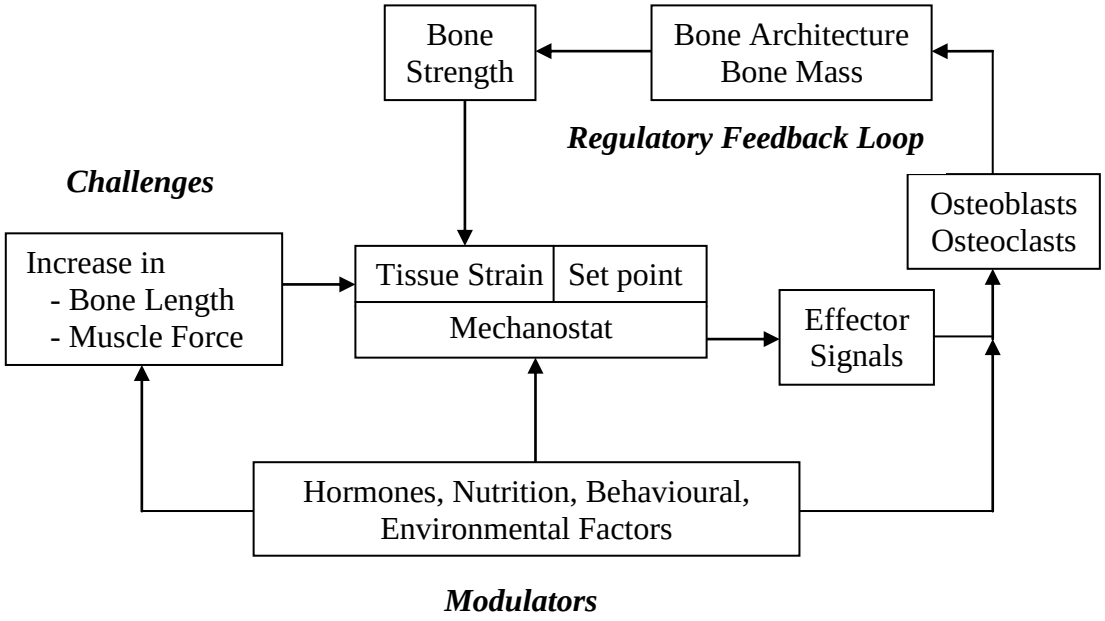

Figure 2.1 The Functional Model of Bone Development: the developing bone – slave or master of its cells and molecules? (from Rauch & Schöenau, 2001).

Muscles cause the largest loads on bones and bone strains that control the biological mechanisms that determine whole-bone strength. Mechanical loads and maximal strains acting on bone must be of sufficient intensity, greater than those of normal everyday living, to promote increases in skeletal mass during growth (Scott et al., 2008). Some of the largest loads placed on the skeleton are physiological and result from muscle contractions. Research has demonstrated a strong relationship between bone properties and muscle development in children, which suggests that increasing muscle mass during development can create a stimulus large enough to increase bone mass, and in turn bone strength (Klentrou, 2016; Rauch et al., 2004; Schöenau & Frost, 2002). Correlations between lean body mass and bone mineral content have been found during growth (Faulkner et al., 1993; Manzoni et al., 1996), with a temporal association between muscle and bone development (Rauch et al., 2004). Research has demonstrated that the peak rate of increase in muscle mass (Rauch et al., 2004)

and, therefore, muscle strength occurs after the age of PHV (Blimkie, 1989), but before the peak accrual of bone mass (Rauch et al., 2004) and bone strength (Jackowski et al., 2009), supporting the *Mechanostat Theory* notion of muscle mass or force driving bone strength. However, a temporal relationship has yet to be determined because both bone and muscle development may be affected by a third factor (e.g., growth and maturation). As the temporal accrual of muscle mass and bone mass is timed with peak height velocity, it is likely that maturity influences the development of the functional muscle-bone unit. Our longitudinal study will try to ascertain the effect of maturity on changes in bone and muscle strength, and in turn its influence on the inter-related relationship between muscle and bone during growth by applying a mediated statistical approach.

2.6 Factors Affecting the Functional Muscle-Bone Unit

It has already been mentioned that bone development is a complex interaction of various factors such as nutritional, hormonal and mechanical stimuli (Gordon, 2003; Steelman & Zeitler, 2001), which are factors commonly affecting muscle development. These factors must therefore be discussed looking at muscle and bone as a functional system, a functional muscle-bone unit.

2.6.1 Muscle Development

It is well known that muscle mass and bone mass are closely associated (Doyle et al., 1970). The correlation between lean body mass (LBM), which is often considered a surrogate for skeletal muscle mass, and BMC is especially close during growth and maturation (Manzoni et al., 1996; Wolfe et al., 2006). The M*echanostat Theory* postulates that the

statistical association between LBM and BMC reflects a direct cause and effect relationship, (Frost, 2000). According to this hypothesis, the skeleton continually adapts its strength to the loads to which it is exposed in order to keep bone deformation within safe limits. The largest physiological loads on the skeleton result from muscle contraction, which puts several-fold larger stresses on the skeleton than the simple effect of gravity (Burr, 1997).

During these early years, patterns of diet and physical activity begin forming and are carried into adulthood. Poor nutritional and physical activity habits may result in one not achieving his/her full potential in terms of peak bone mass and muscle strength. (Pollitzer et al., 1989).

2.6.2 Physical Activity and the Muscle-Bone Unit

Partitioning the effects of physical activity on lean mass accrual from normal growth and maturation, however, presents a major research challenge (Baxter-Jones et al., 2005). In the Saskatchewan Pediatric Bone Mineral Accrual Study, researchers repeatedly assessed one hundred nine boys and one hundred thirteen girls for 6 years (Baxter-Jones et al., 2003, 2008). Participants were 8–15 years old at entry and measurements such as stature, body mass, and physical activity were assessed biannually. Body composition was assessed annually by DXA and physical activity was determined using standardized questionnaires for children and adolescence. The results of this longitudinal study have demonstrated the independent effects of physical activity on total body and regional lean mass accrual, while accounting for the confounding effects of growth and maturation. These findings suggest the importance of physical activity during the adolescent growth period on lean mass accrual (Baxter-Jones et al., 2003, 2008). That finding was important, since adolescence represents not only the period

of the lifespan when physical activity levels decrease substantially (Sallis et al., 2000, Sherar et al., 2007, Trost et al., 2002), but also a time when substantial changes in body composition are occurring.

Thus, the incorporation of good exercise habits during childhood and adolescence might provide important stimuli for gaining and maintaining bone mass (Ruiz et al., 1995; Snow, 1996; Turner et al., 1992). In adults, some studies have shown increases in BMD with exercise (Nichols et al., 1994; Snow-Harter et al., 1992) whereas others have shown decreases (Rockwell et al., 1990) or no change (Bassey et al., 1998). The reasons for the disparity are unclear, although the intensity of the training program, the type of activity used, and/or nutrition factors may have all played a role (Snow, 1996; Lanyon et al., 1996). In younger children, studies in the area of bone accretion through activity and exercise have generally shown positive results, with most studies showing gains in bone mass with exercise (Bradney et al., 1998; Morris et al., 1997).

It is well documented that physical activity increases lean body mass (LBM) and muscle strength in children and adolescents. Increases in muscle strength and neuromuscular adaptations have been observed after various strength training interventions in youth (Faigenbaum et al, 1999; Ozmun et al., 1994; Pfieffer & Francis, 1986; Ramsay et al., 1990; Sadres et al., 2001). In addition, there have been a number of reviews demonstrating positive effects of resistance training in children and adolescents (Blimkie, 1992; Blimkie, 1993; Falk and Tenenbaum, 1996; Malina, 2006). In turn, there is evidence suggesting that LBM is a major contributor to BMC, BMD and bone microstructure (El Hage et al., 2009; Far et al., 2014; Gracia-Marco et al., 2012; Pietrobelli et al., 2002). Previous longitudinal studies have

also demonstrated that physically active children exhibit greater increases in both LBM and bone mass than their sedentary counterparts (Slemenda et al., 1991, 1994).

During growth, therefore, exercise can act directly through mechanical loading and indirectly through endocrine regulation to influence bone modeling and bone geometry (Klentrou, 2016). The latter, i.e., the endocrine influence is not a subject of this review, thus it will not be discussed. In regards to the former, i.e. mechanical loading, results indicate bone mass to be particularly responsive to exercise programs early in puberty with the magnitude of effect decreasing in post-pubertal years, insinuating there is a *window of opportunity* for bone response (MacKelvie et al., 2002). Furthermore, habitual physical activity has been shown to enhance not only bone accrual (Baxter-Jones et al., 2003) in youth, but lean mass (Baxter-Jones et al., 2008) as well; both of which are important to promote musculoskeletal health and function in older age (Lefevre et al., 1990). Thus, physical activity can affect the muscle-bone unit as a system such that physical activity may affect muscle, which in turn, affects bone. Regarding specifically the effects on bone, the next chapter presents a systematic review of the literature on the effects of physical activity and exercise training programs on bone accrual in children.

2.6.3 Nutrition and the Muscle-Bone Unit

Adequate nutrition is important for both muscle and bone development. Total energy, protein, calcium and vitamin D intake are key nutritional factors that may act directly or indirectly on muscle and bone (Bass et al., 2005). Calcium and vitamin D are both nutritional factors that are important to the promotion of skeletal health and growth. Calcium is a major constituent of bone and dietary calcium is thought to be an important determinant in

maximizing bone mineral acquisition during growth (Bass et al., 2005; Cadogan et al., 1997; Valimaki et al., 1994). Calcium requirement increases during periods of rapid growth, and vitamin D is an important complement of calcium as it facilitates the absorption of calcium from the diet. Therefore, a concern with malnutrition is that vitamin D deficiency could cause an increase in the bone remodeling rate, which could potentially impair bone accretion during skeletal growth (Gordon, 2003).

Muscle and bone are negatively impacted when there is nutritional deficiency, specifically when there is protein and energy malnutrition (Bass et al., 2005). Energy deficiency affects bone strength by altering mechanostatic set points by creating hormonal imbalances (Bass et al., 2005; Cobb et al., 2003), while protein and caloric restriction can result in delayed skeletal growth and reduced bone mass (Bass et al., 2005; Bonjour & Rizzoli, 1995). In addition, energy and more so protein deficiency may also reduce muscle mass, which in turn can diminish the mechanical demands placed on bone by muscle (Bass et al., 2005a and 2005b). However, protein deficiency rarely occurs without energy deficits. The biochemical pathway by which protein or energy deficiency have catabolic effect on bone growth and development is likely by suppressing IGF-1 levels or bone cells' sensitivity to IGF-1 (Bass et al., 2005). IGF-1 is a nutritionally dependent bone trophic factor that is critical during periods of bone mineral accrual and low levels have been associated with poor bone health in energy deficient adolescent females (Soyka et al., 1999). Moreover, IGF-1 can act as a myokine as it is secreted by muscle contractions and can have a potentially IGF-1 mediated paracrine signaling mechanism at the muscle-bone interface by linking muscle hypertrophy with bone anabolism (Hamrick, 2011). Therefore, energy deficiency can affect bone directly through the actions of IGF-1, and indirectly through its negative impact on muscle strength.

CHAPTER 3

Physical Activity Interactions with Bone Accrual in Children and Adolescents – a Systematic Literature Review[1]

3.1 Introduction

The use of physical activity in maintaining bone health throughout the lifespan and ultimately preventing osteoporosis has been the focus of considerable research in improving peak bone mass (PBM) in order to minimize later bone loss (Beck & Snow, 2003). It is generally accepted that engaging in physical activity during growth enhances bone development (Boot et al., 1997; Janz et al., 2001; Janz et al., 2006). Habitual physical activity has been shown to enhance lean mass (Baxter-Jones et al., 2008) and bone accrual (Baxter-Jones et al., 2003) in youth, both of which are believed to promote bone health and muscle function in older age (Lefevre et al., 1990). Furthermore, 'when' activity occurs during the lifespan is important as physical activity at a young age can account up to 17% of the variance in bone mineral density (BMD) seen in individuals in their late 20s (Davies et al., 2005).

[1]Modified from Ludwa IA, and Klentrou P. Physical activity interactions with bone accrual in children and adolescents. In: Y. Dionyssiotis (Ed.), *Osteoporosis*. InTech Open Access Publishers, pp. 379-408, 2012.

In addition to the timing of physical activity, the method by which activity imparts its benefits on bone is also important. Mechanical loading of sufficient intensity to promote increases in skeletal mass during growth requires maximal strains to be greater than those of normal everyday living. If the bone is properly overloaded, the load will elicit a modeling response, which makes the bone susceptible to new levels of mechanical demand (Bailey et al., 1996). Some of the largest loads placed on the skeleton are physiological ones resulting from muscle contractions (Rauch et al., 2004; Scheonau & Frost, 2002). Furthermore, gravitational or ground reaction forces are also capable of generating the loads necessary to elicit a favourable response in bone. These two loading methods have lead to investigations of bone responses to different forms of activity with comparisons between athletes and non-athletes. Studies have demonstrated young athletes involved in high-impact, weight-bearing activities such as gymnastics and running have higher BMD (Lehtonen-Veromaa et al., 2000b, 2000c) than athletes participating in low-impact sports such as swimming (Bellew and Gehrig, 2006; Cassell et al., 1996; Courteix et al., 1998). Resistance training and simple jumping exercises have also been shown to have positive effects on femoral BMD in adolescent females and as such can be useful in promoting bone growth and maintaining acquired gains (Fuchs and Snow, 2002; Kato et al., 2006; Nichols et al., 2001). Therefore, different forms of physical activity, such as resistance training (Nichols et al., 2001) and weight-bearing exercise (Fuchs & Snow, 2002; Lehtonen-Veromaa et al., 2000c) have been shown to have positive effects on the developing skeleton through ground reaction forces and muscle contractions.

Various studies have examined the relationship between physical activity and markers of bone metabolism (Creighton et al., 2001; Lehtonen-Veromaa et al., 2000a). Little research, however, has been conducted on markers of bone formation and resorption in relation to

different types of sports, particularly in children and adolescents. In female athletes between the ages of 18-26, Creighton et al. (2001) found bone formation to be lower and resorption similar in swimmers compared to basketball, volleyball, and soccer players. In a younger population of boys and girls, ages 9-16 years, no differences were found in any markers of bone metabolism between gymnasts (Lehtonen-Veromaa et al., 2000a), swimmers (Derman et al., 2008) and controls. Therefore, research investigating the relationship regarding bone markers and different activity types is limited, but even more so in children and adolescents, making it difficult to ascertain the effect of sport on bone. The examination of biochemical measurements of bone turnover, in addition to static measures of bone, is advantageous in the study of skeletal metabolism and growth as they provide an understanding of the dynamic course of bone remodelling. To date, the use of biochemical marks of bone turnover in physical activity interventions on bone in youth has been extremely limited.

Difficulties in comparing and assessing the benefits of physical activity on bone during growth reflect the varying methodologies used between studies. Physical activity interventions aimed at improving bone health in youth have been subject to limited maturational comparisons as the majority of interventions have been conducted in one distinct maturational group. Furthermore, the types of physical activity interventions that have been applied have varied greatly between studies. Discrepancies in results are due in part to the varying bone assessment techniques that are used across cross-sectional and intervention studies. Many of the aforementioned studies measured improvements in BMD using dual-energy x-ray absorptiometry (DXA). The use of DXA to interpret and evaluate BMD in the growing years can be difficult as there are considerable changes to the size and shape of bone (Bailey et al., 1996; Gordon, 2003; Schöenau et al., 2004). Furthermore, the measurements provided by

DXA fail to account for the architecture, organization of tissues, mechanical properties and other factors known to impart bone strength. In addition, the bone assessment techniques used in majority of these studies have provided a static rather than dynamic picture of bone, which could in fact allow for more comparisons across studies.

Evidence supporting the role of physical activity on bone health has accumulated from a wide range of studies investigating different activity methods using athletes, non-athletes and inactive individuals. Although these studies contribute to the literature, they do not provide us with direct evidence that physical activity does impart benefits to bone health. In response, there has been an increase in the number of intervention studies conducted, particularly in the school setting. Schools provide an ideal setting for physical activity interventions as they allow for a large population of children and adolescents, regardless of socioeconomic status, to be targeted in a somewhat controlled environment, where they already spend a majority of their day during their most skeletally responsive years (Hughes et al. 2007).

The primary objective of this chapter is to conduct a systematic review on the effectiveness of exercise/physical activity interventions to improve bone accrual and bone properties in children and adolescents. Key findings from controlled intervention trials using various techniques to assess bone mineral density, content and strength changes will be discussed and be grouped according to maturity status. This will help to shed light on the best time during growth and development to influence bone health and to ascertain if there is indeed a window of opportunity for bone response. We will also discuss and compare the different types of interventions used to affect changes in bone properties in youth, to determine if there is a modality that is best suited to improving bone development and to what

degree these interventions influence changes in bone. Furthermore, we will address the characteristics of loading that have been shown to be best associated with particular structural improvements as interventions can be designed to impart mechanical loading on bone either by jumping or by resistance training where the weight-bearing load on bone is applied through muscle. As the majority of interventions measure only static properties of bone, this chapter will also be used to discuss bone remodelling parameters influenced by exercise interventions. To our knowledge, there have not been any studies examining the effects of physical activity interventions on markers of bone remodelling in children and adolescents.

3.2 Methods

3.2.1 Eligibility Criteria and Search Strategy

The aim of the literature search was to find all available randomized control trials and controlled studies that examined the effects of any type of exercise or physical activity intervention trial on bone status in healthy (non-clinical, non-athlete) children and adolescents between 6 and 17 years of age. Studies that included calcium interventions in addition to physical activity and exercise were also included. For this review, we included all types of bone parameters from various bone assessment techniques (DXA, pQCT, QUS etc.) as primary outcome measures, provided that there were at least two measurement time points. Primary outcome measures included areal bone mineral density (aBMD), volumetric bone mineral density (vBMD), bone mineral content (BMC), bone area (BA), cortical thickness, bone strength index (BSI), stress-strain index (SSI), maximal moment of inertia (I^{mas}), section

modulus (SM), speed of sound (SOS), broadband ultrasound attenuation (BUA), and markers of bone metabolism.

A computerised search of the MEDLINE and PubMed databases was performed on articles up until 2011 using a comprehensive combination of keywords to describe exercise, bone and participant parameters. The keywords used to describe exercise included: intervention and intervention studies, training, exercise, resistance training, physical education and physical education training, physical activity and motor activity. Bone parameter keywords included: bone mineral, bone density, bone and bones, bone strength, bone accrual and development, bone turnover, resorption, modelling and metabolism. For the participants, keywords such as children, adolescents, boys and girls were used. A total of 2728 studies were found; titles and abstracts were reviewed to determine if they met the inclusion criteria. Papers from all journals were considered and retrieved electronically or by interlibrary loan.

After screening the articles a total of 35 studies met the criteria and were used for the current review. Studies were grouped according to the maturity status of their participants. All studies reported pubertal stages, based on secondary sex characteristics, as described by Tanner (Tanner, 1962). Participants were grouped as either prepubertal (Tanner 1), early pubertal (Tanner 2 and 3), and pubertal (Tanner 4 and 5) to maintain consistency with other literature review groupings. Studies in which authors provided results for more than one maturity group were divided into two parts (A and B).

3.3 Results

Table 3.1 represents the numerical breakdown of all the intervention studies reviewed into particular categories based on the type of intervention that was used and the method in which bone parameters were assessed, as well as the maturity and sex of the population measured. Studies were included more than once if more than one measurement technique was used and if results were separated by sex or maturity group. Table 3.2 is a detailed summary of the design and outcomes of all the physical activity intervention studies reviewed, and are grouped according to the participants' maturity status. The results presented in Table 3.2 express the percentage difference in gain between the experimental groups participating in the intervention in comparison to controls.

Table 3.1 Numerical breakdown by category of physical activity interventions for bone in youth. Prepubertal corresponds to Tanner Stage 1, early pubertal Tanner Stages 2-3, and pubertal Tanner Stages 4-5. Multi pubertal *separate* are studies with results separated by maturity, with *together* being studies that averaged data for more than one maturity group. Boys + girls reflect studies that did not separate results by sex.

Type of Intervention		**Measurement Technique**		**Maturational Status**		**Sex**	
School Based		SXA	1	Prepubertal	16	Boys	12
Part of PE Class	23	DPA	1	Early Pubertal	16	Girls	24
At the School	5	DXA	33	Pubertal	7	Boys + Girls	7
Outside of School	7	HSA	4	Multi Pubertal^{separate}	4		
Jumping	18	pQCT	5	Multi Pubertal^{together}	5		
General WBPA	14	QUS	3				
Resistance Training	3	Bone Markers	1				

PE: physical education; WBPA: weight-bearing physical activity; SXA: single energy x-ray absorptiometry; DXA: dual energy x-ray absorptiometry; DPA: dual photon absorptiometry; pQCT; peripheral QCT; HSA: hip structural analysis; QUS: quantitative ultrasound.

The majority of the intervention studies were school-based, with 23 of the studies being conducted as part of a regular physical education class and 5 of the studies being conducted outside the physical education class at some point within the school day. Approximately half (51%) of the studies utilized specific jumping interventions that relied on ground reaction forces in order to elicit a positive response on bone. Fourteen studies consisted of general weight-bearing types of activities such as running, volleyball, aerobics etc., with only 3 studies specifically using resistance training with free or machine assisted weights. Sixteen jumping interventions, 14 WBPA interventions, and one resistance training study reported significant increases in their primary bone outcomes. These results translated into 79.5% of physical activity interventions positively influencing some form of bone parameter in children and adolescents. Furthermore, five studies also included calcium interventions, which demonstrated benefits to bone in addition to physical activity.

Of the studies reviewed, 17 presented results for girls, seven for boys, with 11 studies presenting data for boys and girls together. Sixteen studies conducted interventions in each of the prepubertal and early pubertal groups. The smallest number of studies (n=7) was performed in pubertal youth. The majority of the pubertal interventions were completed on a population of girls, with only one study (Weeks et al., 2008) including boys in their sample. An even number of boys and girls were represented in the results of prepubertal youth, with 8 studies separately reporting results for boy and girls and 2 grouping results together. In early pubertal children, a greater number of studies were conducted on and included girls. Ten studies reported results separately for girls, 3 for boys and 5 did not distinguish results between sexes.

DXA was the measurement technique predominantly used (94%) to assess bone, followed by pQCT (14%) and then QUS (8.5%). In total, five studies used more than one technique to determine changes in bone and these were all done in conjunction with DXA measurements. Four studies using DXA also performed hip structural analysis (HSA). HSA is an application for DXA that allows for the estimation of geometric contributions to bone strength in the proximal femur, which may potentially provide a good representation of bone strength (Bonnick, 2007). Our literature search found one study (Schneider et al., 2007) that measured serum markers of bone formation and resorption in adolescents. As static measures require longer durations for differences to be found, measuring biochemical markers of bone turnover would allow for changes in dynamic properties of bone to be detected sooner.

Table 3.2 Intervention studies on the effects of exercise on bone indices in youth.

Reference	Population	Intervention	Measures	Results	Limitations
Pre Pubertal (Tanner Stage 1)					
Alwis et al. (2008a)	Boys, White Ex: n=80, Con: n=57 Age range: 6.7-9 yrs All remained TS 1 Randomized by school: 1 Ex + 3 Con	24 Months Typical PE class: ball games, running jumping, climbing Ex: 40min/day (200min/wk) Con: 60min/wk Compliance: Con 84%, Ex 95%	DXA BMC: total body and L3 vertebra L3 vertebral width HSA of femoral neck	BMC L3: +3% L3 width: +1.3%	Uneven sample size between Ex and Con. Accelerometers captured only 4 days of 2-yr intervention Compliance not reported
Alwis et al. (2008b)	Girls, White Ex: n=53, Con: n=50 Age range: 6.7-9 yrs All remained TS 1 Randomized by school: 1Ex + 3 Con	12 Months Typical PE class: ball games, running jumping, climbing Ex: 40min/day (200min/wk) Con: 60min/wk Compliance: Con 76%, Ex 95%	DXA and HSA BMC, aBMD, periosteal and endosteal diameter, cortical thickness, CSMI section modulus, and CSA of FN	No significant between group differences were found	Follow up periods varied Higher spare time activities in control group.
Bass et al. (2007)	Boys, White + Asian Total n=88, 7-11 yrs Ex Placebo: n=21 Ex Ca: n=20 No Ex Ca: n=21 No Ex Placebo: n=26 Randomized groups Ca: double blind	8.5 Months Part of PE class: 20min 3x week Hopping jumping, skipping moderate or low impact Ex: Ground rx forces 2-8 x BW No Ex: Ground rx forces 1 x BW Ca: 800mg Ca/day Compliance 86%	DXA BMC: total body, lumbar spine, femur, tibia-fibula, humerus, radius-ulna	Femur BMC: +2% Ex+Ca > all other grps Tibia-fibula BMC: +2% ExCa>Ex Placebo +3% Ex Ca> No ExCa and No Ex Pl NS for BMC in arms	Low sample sizes in each of the groups Control grp participated in low impact exercise making possible differences between groups smaller Population not all TS1 61% TS 1, 39% TS 2
Bradney et al. (1998)	Boys, White N=20 Ex, m=20 Con Age range: 8.4-11.8 All remained TS 1 Randomized by school: 1 Ex + 1 Con	8 Months Program outside of school: aerobics, soccer, volleyball, dance, gymnastics, basketball, weight training 30 minutes, 3 x week	DXA aBMD: total body and lumbar spine, femur, Femoral Midshaft BMC aBMD and vBMD, and cortical thickness	aBMD TB: +1.2% aBMD LS: +2.8% BMC and aBMD femoral midshaft: +5.6% cortical thickness: +6.4%	Low sample sizes in each of the groups volumetric bone densities were derived/estimated

Ex: exercise group; Con: control group; BMC: bone mineral content; aBMD: areal bone mineral density; vBMD: volumetric BMD; BA: bone area; BMAD: bone mineral apparent density (BMD adjusted for BA); TB: total body; LS: lumbar spine; FN: femoral neck; NN: narrow neck; PF: proximal femur;, WT: wards triangle; TR: trochanter; SXA: single energy x-ray absorptiometry; DXA: dual energy x-ray absorptiometry; DPA: dual photon absorptiometry; QCT: qualitative computed tomography, pQCT; peripheral QCT; HSA: hip structural analysis; QUS: quantitative ultrasound; SOS: speed of sound; SM: section modulus; CSMI: cross-sectional moment of inertia; IBS/BSI: index of bone structural strength; SSI: strength strain index; BUA: broadband ultrasound attenuations; Ca: calcium; Rx: reaction; BW: body weight; PE: physical education; TS: Tanner stage; Pl: Placebo; Grps: Groups; NS: no significant.

Table 3.2 Continued - Intervention studies on the effects of exercise on bone indices in youth.

Reference	Population	Intervention	Measures	Results	Limitations
Pre Pubertal (Tanner Stage 1)					
Fuchs et al. (2001)	Boys and Girls, Asian and White Age range: 5.9-9.8 yrs n=45 Ex., n=41 Con Randomized 1 school All remained TS 1	7 Months Activities added to PE classes: 10 min 3x week jumping 50-100 high box jumps, 2 footed Ground rx forces = 8.8 x BW 90% Compliance	DXA BMC and aBMD: lumbar spine and femoral neck BA: femoral neck	BMC LS: +3% BMC FN: +4.5% aBMD LS: +2% aBMD FN: NS BA FN: +2.9%	cannot distinguish results between boys and girls
Hassel-strom et al. (2008)	Boys and Girls, White (Ex: n= 135 and 108) (Con: n= 62 and 76) Age Range: 6-8 No Randomization TS 1 and 2	36 Months School based curriculum, time increased: 4 classes 180 min/wk Con: regular school curriculum 90min/wk Activities conducted in classes not mentioned	Peripheral DXA BMC and BMD: Calcaneus and distal forearm	Girls: NS changes in calcaneal and distal forearm BMD BMC forearm: +12.5% forearm area: +13.2% Boys: NS changes in all measures	Non-randomized study design allowing for selection bias DXA locations measured less studied Possible differences in standard anatomical region measured due to growth
Linden et al. (2006)	Girls, White Ex: n=49, Con: n=50 Age range: 7-9 All remained TS 1 Randomized by school: 1 Ex + 3 Con.	24 Months Typical PE class: ball games, running jumping, climbing Ex: 40min/day (200min/wk) Con: 60min/wk Ex. Attendance: 90%	DXA BMC and aBMD: TB, LS L2-L4, L3 FN, and Leg vBMD, bone size: L3 and FN	BMC: L2-L4 +3.8%, L3 +7.2%, Leg +3.0% aBMD: TB +0.6%, L2-L4 +1.2%, L3 +1.6%, Leg +1.2% Bone Size: L3 +1.8%, and FN +0.3%	Differences in leisure time PA Compliance not reported
Linden et al. (2007)	Boys, White Ex: n=81, Con: n=57 Age range: 7-9 All remained TS 1 Randomized by school: 1 Ex + 3 Con.	12 Months Typical PE class: ball games, running jumping, climbing Ex: 40min/day (200min/wk) Con: 60min/wk Ex. Attendance: 90%	DXA BMC and aBMD: TB, L3 vertebra, FN Bone Width: L3 and FN	BMC, aBMD, bone width L3: +5.9%, +2.1% and +2.3%	Uneven sample size between Ex and Con. Compliance in Con Low Only assessed duration of PA, not intensity or effort

Ex: exercise group; Con: control group; BMC: bone mineral content; aBMD: areal bone mineral density; vBMD: volumetric BMD; BA: bone area; BMAD: bone mineral apparent density (BMD adjusted for BA); TB: total body; LS: lumbar spine; FN: femoral neck; NN: narrow neck; PF: proximal femur;, WT: wards triangle; TR: trochanter; SXA: single energy x-ray absorptiometry; DXA: dual energy x-ray absorptiometry; DPA: dual photon absorptiometry; QCT: qualitative computed tomography, pQCT; peripheral QCT; HSA: hip structural analysis; QUS: quantitative ultrasound; SOS: speed of sound; SM: section modulus; CSMI: cross-sectional moment of inertia; IBS/BSI: index of bone structural strength; SSI: strength strain index; BUA: broadband ultrasound attenuations; Ca: calcium; Rx: reaction; BW: body weight; PE: physical education; TS: Tanner stage; Pl: Placebo; Grps: Groups; NS: no significant.

Table 3.2 Continued - Intervention studies on the effects of exercise on bone indices in youth.

Reference	Population	Intervention	Measures	Results	Limitations
Pre Pubertal (Tanner Stage 1)					
Macdonald et al. (2007) (Part A)	Boys and Girls Asian and White Ex: n=140, Con: n=72 Age range: 9.6-10.8 Randomized by school: 7 Ex. + 3 Con.	16 Months Ex: 15 min/day PA 5 x week, 5-36 jumps/day 4 x week Con: regular school curriculum Compliance 74%	pQCT BSI distal tibia SSI tibial midshaft	Boys: BSI distal tibia increased ~+25% Girls: NS changes in all measures	Low Compliance Potential bias for school selection Low Compliance Uneven sample sizes and distribution of sexes
MacKelvie et al. (2001) (Part A)	Girls, White + Asian Ex: n=44, Con: n=26 Age range: 9.4-10.6 Randomized by schools: 7 Ex + 7 Con	7 Months Activity added to regular PE class: 10min, 3 x week 50-100 jumps and circuit training, progressing w/jumps Jumping = 3.5-5 x BW Compliance 80% across schools	DXA BMC and aBMD: TB, LS, PF, FN vBMD: FN	NS differences in any of the bone variables measured	vBMD measurements were derived/estimated Uneven sample size between Ex and Con. More Ex's advances from TS 1 to TS2
MacKelvie et al. (2002)	Boys White + Asian Ex: n=61, Con: n=60 Age range: 9.7-10.9 Randomized by schools: 7 Ex + 7 Con	7 Months Activity added to regular PE class: 10min, 3 x week 50-100 jumps and circuit training, progressing w/jumps Jumping = 3.5-5 x BW Compliance 80% across schools	DXA BMC and aBMD: TB, LS, PF, FN vBMD: FN	BMC TB: +1.6% aBMD PF: +1%	vBMD measurements were derived/estimated
MacKelvie et al. (2004)	Boys, White + Asian Ex: n=31, Con: n= 33 Age range: 9.6-10.7 Randomized by schools: 7 Ex + 7 Con	20 Months Activity added to regular PE class: 10min, 3 x week 50-100 jumps and circuit training, progressing w/jumps Jumping = 3.5-5 x BW	DXA and HSA BMC and BA: TB, LB, PF, FN, and TR HAS: PF, NN, TR , FN SM: FN	BMC FN: +4.3% Cross-sectional moment of inertia: +12.35% SM: +7.4%	Study compliance: Ex 39% And Con 42% More Con remained TS 1 and more Ex's advanced to TS 3

Ex: exercise group; Con: control group; BMC: bone mineral content; aBMD: areal bone mineral density; vBMD: volumetric BMD; BA: bone area; BMAD: bone mineral apparent density (BMD adjusted for BA); TB: total body; LS: lumbar spine; FN: femoral neck; NN: narrow neck; PF: proximal femur;, WT: wards triangle; TR: trochanter; SXA: single energy x-ray absorptiometry; DXA: dual energy x-ray absorptiometry; DPA: dual photon absorptiometry; QCT: qualitative computed tomography, pQCT; peripheral QCT; HSA: hip structural analysis; QUS: quantitative ultrasound; SOS: speed of sound; SM: section modulus; CSMI: cross-sectional moment of inertia; IBS/BSI: index of bone structural strength; SSI: strength strain index; BUA: broadband ultrasound attenuations; Ca: calcium; Rx: reaction; BW: body weight; PE: physical education; TS: Tanner stage; Pl: Placebo; Grps: Groups; NS: no significant.

Table 3.2 Continued - Intervention studies on the effects of exercise on bone indices in youth.

Reference	Population	Intervention	Measures	Results	Limitations
Pre Pubertal (Tanner Stage 1)					
McKay et al. (2000)	Boys and Girls White and Asian Ex: n=63, C: n=81 Age range: 6.9-10.2 School randomized	8 Months Part of PE classes: jumping, hopping, skipping 2 x week 3 x week 10 tuck jumps Con: regular PE classes	DXA aBMD: TB, LS, PF, FN, TR	aBMD TR: +1.2%	All boys remained TS 1, with some girls maturing to TS 2 Compliance not reported
Petit et al. (2002) (Part a)	Girls, Asian + White Age range: 9.4-10.6 Ex: n=43, Con: n=25 Randomized by schools: 14 schools ethnic stratification	7 Months Part of PE classes: 10-12 min 3x week: 5 x diverse jumping exercise stations Con: regular PE classes Ground rx forces=3.5-5 x BW	DXA and HSA abed: TB, LS, TR, PF cortical thickness, area and SM: PF	NS differences in any of the bone variables measured	Compliance not reported Errors related to method of measurement
Valdimar-sson et al. (2006)	Girls, White Ex: n=53, Con: n=50 Age range: 7-9 yrs Ex group come from one school	12 Months Typical PE class: ball games, running jumping, climbing Ex: 40min/day (200min/wk) Con: 60min/wk. 90% Attendance	DXA BMC and aBMD: TB, LS (L2-L4), L3, FN, and leg vBMD: L3 and FN	BMC LS: +4.7% BMC L3: +9.5% aBMD LS: 2.8% aBMD L3: 3.1% Bone width L3: +2.9%	No randomization Compliance low in controls volumetric bone densities were derived/estimated
Van Lang-endonck et al. (2003)	Girls Ethnicity not reported Ex: n=21, Con: n=21 21 pairs of monozy-gotic twins Age range: 8-9yrs	9 Months Ex: 3x week: hopping/jumping Progression: removal of shoes different stimulus Ground rx forces not measured Compliance: Ex 91%	DXA BMC, aBMD, BA: FN and PF	BMC PF: +2.5% aBMD PF: +1.3% BMC FN: +2.0% aBMD FN: +2.4%	Some of the girls participated in high impact sports during their leisure time - separate analysis conducted

Ex: exercise group; Con: control group; BMC: bone mineral content; aBMD: areal bone mineral density; vBMD: volumetric BMD; BA: bone area; BMAD: bone mineral apparent density (BMD adjusted for BA); TB: total body; LS: lumbar spine; FN: femoral neck; NN: narrow neck; PF: proximal femur;, WT: wards triangle; TR: trochanter; SXA: single energy x-ray absorptiometry; DXA: dual energy x-ray absorptiometry; DPA: dual photon absorptiometry; QCT: qualitative computed tomography, pQCT; peripheral QCT; HSA: hip structural analysis; QUS: quantitative ultrasound; SOS: speed of sound; SM: section modulus; CSMI: cross-sectional moment of inertia; IBS/BSI: index of bone structural strength; SSI: strength strain index; BUA: broadband ultrasound attenuations; Ca: calcium; Rx: reaction; BW: body weight; PE: physical education; TS: Tanner stage; Pl: Placebo; Grps: Groups; NS: no significant.

Table 3.2 Continued - Intervention studies on the effects of exercise on bone indices in youth.

Reference	Population	Intervention	Measures	Results	Limitations
Early Pubertal (Tanner Stage 2-3)					
Barbeau et al. (2007)	Girls, Black n=77 Ex., n=83 Con. Age range: 8-12 yrs Recruited from 8 elementary schools	10 Months After school intervention 5 days/week, 80 min PA: 25min skills, 35min MVPA, 20min toning + stretching	DXA Total body BMD, BMC	BMC TB: +4.0% BMD TB: +2%	Examined girls who attended 40% of classes 2d/wk Main focus was to improve cardiovascular fitness Low compliance
Courteix et al. (2005)	Girls, White (n=85) Age range: 8-13 yrs Ex Ca: n=12 Ex Placebo: n=42 No Ex Ca: n=10 No Ex Placebo: n=21 Randomized, Blinded	12 Months Ex: 7.2h/week No Ex: 1.2h/week Ca: 800 mg/day Compliance 75% Ex: Participated in weight bearing physical activity	DXA aBMD: TB, LS, FN, WT	aBMD TB: +6.3% aBMD LS: +11% aBMD FN: +8.2% aBMD WT: 9.3% (all Ex Ca > No Ex Pl) NS differences between other groups	Uneven sample size distribution between groups Type of exercise not controlled Exercise based on habitual activity
Heinonen et al. (2000) (Part A)	Girls, White Ex: n=25, Con:, n=33 Age range: 10-12yrs Selection to groups decided by teachers	9 Months Step aerobic program: 50 min 2 x week: 20 min of jumping exercises: 100-200 jumps from box (two and one footed) Ground rx forces not measured Compliance: Ex 73%, Study 92%	DXA and pQCT BMC: LS, FN, and TR Cortical area: tibial midshaft	BMC LS: +3.3% BMC FN: +4.0%	Compliance low Potential selection bias due to teachers selecting groups
Iuliano-Burns et al. (2003)	Girls, White + Asian Total n=64 Age range: 8-9 yrs Mod Ex. Ca: n=16 Mod Ex. Pl: n=16 Low Ex. Ca: n=16 Low Ex. Pl: n=16 Randomized groups	8.5 Months Ex: 20 min 3 x week Mod Ex. Impact: skipping, hopping, jumping. Used hand weights in final 8 weeks Low Ex. Impact: stretching Ca: average of 434 mg/day Compliance: Ex 93%, Study 88%	DXA BMC: LS, Femur, Tibia-Fibula	BMC tibia-fibula: +3% Mod ex>Low Ex. +7.1% Mod Ex Ca > Low Ex. No Pl.	Low sample sizes

Ex: exercise group; Con: control group; BMC: bone mineral content; aBMD: areal bone mineral density; vBMD: volumetric BMD; BA: bone area; BMAD: bone mineral apparent density (BMD adjusted for BA); TB: total body; LS: lumbar spine; FN: femoral neck; NN: narrow neck; PF: proximal femur;, WT: wards triangle; TR: trochanter; SXA: single energy x-ray absorptiometry; DXA: dual energy x-ray absorptiometry; DPA: dual photon absorptiometry; QCT: qualitative computed tomography, pQCT; peripheral QCT; HSA: hip structural analysis; QUS: quantitative ultrasound; SOS: speed of sound; SM: section modulus; CSMI: cross-sectional moment of inertia; IBS/BSI: index of bone structural strength; SSI: strength strain index; BUA: broadband ultrasound attenuations; Ca: calcium; Rx: reaction; BW: body weight; PE: physical education; TS: Tanner stage; Pl: Placebo; Grps: Groups; NS: no significant.

Table 3.2 Continued - Intervention studies on the effects of exercise on bone indices in youth.

Reference	Population	Intervention	Measures	Results	Limitations
Early Pubertal (Tanner Stage 2-3)					
MacKelvie et al. (2001) (Part B)	Girls, White + Asian Ex: n=43, Con: n=64 Age range: 9.9-11.1 yr Randomized by schools: 7 Ex + 7 Con	7 Months Part of PE class: 10min 3x week 50-100 jumps and circuit training, progressing w/jumps Jumping = 3.5-5 x BW Compliance 80% across schools	DXA BMC and aBMD: TB, LS, PF, FN Volumetric BMD: FN	BMC LS +1.8% aBMD LS +1.7% BMC FN: NS aBMD FN: +1.6% vBMD FN: +3.1%	Volumetric bone densities were derived/estimated Uneven sample size between Ex and Con.
MacKelvie et al. (2003)	Girls, Asian + White Ex: n=33, C: n=43 Age range: 9.3-10.7 Randomized by schools: 7 Ex + 7 Con	20 Months Part of PE class: 10min 3x week 50-100 jumps and circuit training, progressing w/jumps Jumping = 3.5-5 x BW Compliance 42% over 20 Mos.	DX BMC: LS and FN	BMC LS: +3.7% BMC FN: +4.6%	Con group older and more mature Compliance not reported for Ex. Group
Macdonald et al. (2007) (Part B)	Boys and Girls Asian and White Ex: n=135, Con: n=57 Age range: 9.6-10.8 yrs Randomized by school: 7 Ex. + 3 Con.	16 Months Ex: 15 min/day PA 5 x week, 5-36 jumps/day 4 x week Con: regular school curriculum Compliance 74%	pQCT BSI distal tibia SSI tibial midshaft	NS changes in any of the measures	Low Compliance Potential bias for school selection Uneven sample sizes and distribution of sexes between groups
Macdonald et al. (2008)	Boys and Girls Asian and White Ex: n=140, Con: n=72 Age range: 9-11 yrs Randomized by school: 7 Ex. + 3 Con. TS 1-3	16 Months Ex: 15 min/day PA 5 x week, 5-36 jumps/day 4 x week Con: regular school curriculum Compliance 74%	DXA and HSA FN bone strength, geometry, and BMC BMC: TB, PF, LS	Boys: BMC LS: +2.7% BMC TB: +1.7% Girls: section modulus of FN: +5.4% (only in girls with 80% compliance)	Low teacher compliance Uneven sample sizes and distribution of sexes btw grps More boys prepubertal and girls early pubertal Results not separated by maturity status

Ex: exercise group; Con: control group; BMC: bone mineral content; aBMD: areal bone mineral density; vBMD: volumetric BMD; BA: bone area; BMAD: bone mineral apparent density (BMD adjusted for BA); TB: total body; LS: lumbar spine; FN: femoral neck; NN: narrow neck; PF: proximal femur;, WT: wards triangle; TR: trochanter; SXA: single energy x-ray absorptiometry; DXA: dual energy x-ray absorptiometry; DPA: dual photon absorptiometry; QCT: qualitative computed tomography, pQCT; peripheral QCT; HSA: hip structural analysis; QUS: quantitative ultrasound; SOS: speed of sound; SM: section modulus; CSMI: cross-sectional moment of inertia; IBS/BSI: index of bone structural strength; SSI: strength strain index; BUA: broadband ultrasound attenuations; Ca: calcium; Rx: reaction; BW: body weight; PE: physical education; TS: Tanner stage; Pl: Placebo; Grps: Groups; NS: no significant.

Table 3.2 Continued - Intervention studies on the effects of exercise on bone indices in youth.

Reference	Population	Intervention	Measures	Results	Limitations
Early Pubertal (Tanner Stage 2-3)					
Macdonald et al. (2009)	Boys, Asian + White Ex: m=139, Con: n=63 Age range: 9-11 yrs Randomized by school: 7 Ex. + 3 Con.	16 Months Ex: 15 min/day PA 5 x week, 5-36 jumps/day 4 x week Con: regular school curriculum Compliance 74%	pQCT Second moments of area, cortical area, cortical thickness of tibia	Max second moment of area: +3% Trends for increase in cortical area and thickness, but NS	Uneven sample sizes Higher percentage of TS2 in Ex Group compared to Con at baseline, with Con having more TS1
McKay et al. (2005)	Girls and Boys Asian and White Ex: n=51, Con: n=73 Age Range: 9.5-10.5 No Randomization	8 Months Program: Bounce at the Bell 10 counter movement jumps 3 min 3 x day each school day Ground Rx forces: 5 x BW Compliance: Ex 60%, study 100%	DXA and HSA BMC: PF and TR BA: PF and TR Cortical thickness and area: PF	BMC PF: +2.0% BMC TR: +2.7% BA PF: +1.3% BA TR: +2.0% Con > Ex: BMC and BA TB	Compliance Low Ex group participated in greater PA at baseline Con greater increase in TB BMC and BA
Meyer et al. (2011)	Boys and Girls, White Ex: n=297, Con: n=205 Age range: 6.6-11.7 yrs Randomized by classes: Ex: 16 classes/ 9 schools, Con: 12 classes/6 schools TS 1-3	12 Mos School based program Ex: regular PE class + 2 extra PE classes that include 10 min jumping activities. 2-5min jumping/balancing tasks throughout day Con: regular PE classes	DXA BMC and aBMD: TB, FN, L2-L4	BMC TB: +5.5% BMC FN: +5.4% BMC LS: +4.7% aBMD TB: +8.4% aBMD LS:+7.3% Pubertal stage*group interaction favored prepubertal children	Has distinct pubertal groups but results not separated by maturity. Maturity used to adjust for variables Small sample size of pre pubertal Con grp (loss of data) Compliance not reported
Morris et al. (1997)	Girls, Ethnicity not given, but schools stratified according to ethnicity Ex: n=38, Con: n=33 Age range: 8.6-10.4 yrs No randomization Grouped by teachers	10 Months Activity added to regular PE class: 30 min 3 x week Aerobics, skipping, dance, ball games, progressing to weight training Ground rx forces not measured Compliance: Ex 92%, Study 97%	DXA and BMAD BMC: TB, LS, FN, PF aBMD: TB, LS, PF BMAD: LS, FN	BMC TB and LS: +5.5% BMC FN: +4.5% BMC PF: +8.3% aBMD TB: +2.3% aBMD LS: +3.6% aBMD FN: +10.3% aBMD pF: +3.2% BMAD LS: +2.9%	Potential selection bias as teachers selected groups Maturity greater in Ex than control (due to drop outs) and could contribute to the greater gains seen

Ex: exercise group; Con: control group; BMC: bone mineral content; aBMD: areal bone mineral density; vBMD: volumetric BMD; BA: bone area; BMAD: bone mineral apparent density (BMD adjusted for BA); TB: total body; LS: lumbar spine; FN: femoral neck; NN: narrow neck; PF: proximal femur;, WT: wards triangle; TR: trochanter; SXA: single energy x-ray absorptiometry; DXA: dual energy x-ray absorptiometry; DPA: dual photon absorptiometry; QCT: qualitative computed tomography, pQCT; peripheral QCT; HSA: hip structural analysis; QUS: quantitative ultrasound; SOS: speed of sound; SM: section modulus; CSMI: cross-sectional moment of inertia; IBS/BSI: index of bone structural strength; SSI: strength strain index; BUA: broadband ultrasound attenuations; Ca: calcium; Rx: reaction; BW: body weight; PE: physical education; TS: Tanner stage; Pl: Placebo; Grps: Groups; NS: no significant.

Table 3.2 Continued - Intervention studies on the effects of exercise on bone indices in youth.

Reference	Population	Intervention	Measures	Results	Limitations
Early Pubertal (Tanner Stage 2-3)					
Nemet et al. (2006)	Boys and Girls, Ethnicity not given Ex: n=12, Con: n=12 Age range: 6-16 yrs Obese participants Randomized groups	3 Months Structured activities to mimic PE classes. Mainly endurance: 50% sports, 50% running and games: 1 hour 2 x week Received nutrition counseling	QUS SOS of left tibia	SOS: +2.9% Difference due to significant SOS decrease (-2.6%) in Con, and NS increase in Ex. (+0.6%)	Small sample size Population spans a large age range Compliance not reported
Nichols et al. (2008)	Boys and Girls, White Total n=112 Age range: 9-10yrs Ex only: n=61 Nutrition only: n=9 Ex + nutrition: n=14 Con: n=28 4 schools randomized 85% TS1 at baseline	20 Months Activity added to PE classes: 8-12min 2 x week: of jumping and skipping Ground Rx forces 2-3 x BW Nutrition: 45min biweekly classes to improve Ca intake Compliance: 80% at 8 months, 73% at 20 months	DXA BMD: TB, LS (L2-L4), PF, and FN BMC: TB, LS, FN, PF Measures taken twice: 8 and 20 months	NS differences between groups for any of the bone measurements taken at 8 and 20 months	Uneven sample size distribution between groups TS estimated based on height velocity Leisure PA not controlled: 59% reported participating in organized sports/activities Ground rx forces estimated
Petit et al. (2002) (Part B)	Girls, Asian + White Age range: 9.9-11.1yrs Ex: n=43, Con: n=63 Randomized by schools: 14 schools stratified by ethnic composition	7 Months 10-12 min 3x week 5 x diverse jumping exercise stations Activities done in addition to regular PE classes Con: regular PE classes Ground rx forces=3.5-5 x BW	DXA and HSA aBMD: TR and FN SM: FN cortical thickness: FN	aBMD TR: +1.7% aBMD FN: +2.6% SM FN: +4.0% cortical thickness FN: : +3.2%	Compliance not reported Errors related to method of measurement

Ex: exercise group; Con: control group; BMC: bone mineral content; aBMD: areal bone mineral density; vBMD: volumetric BMD; BA: bone area; BMAD: bone mineral apparent density (BMD adjusted for BA); TB: total body; LS: lumbar spine; FN: femoral neck; NN: narrow neck; PF: proximal femur;, WT: wards triangle; TR: trochanter; SXA: single energy x-ray absorptiometry; DXA: dual energy x-ray absorptiometry; DPA: dual photon absorptiometry; QCT: qualitative computed tomography, pQCT; peripheral QCT; HSA: hip structural analysis; QUS: quantitative ultrasound; SOS: speed of sound; SM: section modulus; CSMI: cross-sectional moment of inertia; IBS/BSI: index of bone structural strength; SSI: strength strain index; BUA: broadband ultrasound attenuations; Ca: calcium; Rx: reaction; BW: body weight; PE: physical education; TS: Tanner stage; Pl: Placebo; Grps: Groups; NS: no significant.

Table 3.2 Continued - Intervention studies on the effects of exercise on bone indices in youth.

Reference	Population	Intervention	Measures	Results	Limitations
Early Pubertal (Tanner Stage 2-3)					
Sundberg et al. (2001)	Boys and Girls, White Ex Boys: n=40 Ex Girls: n=40 Con Boys: n=82 Con Girls: n=66 Age range: 12-16 yrs 2 Schools (1 Ex, 1 Con) Recruited grade 6,7 (12-13yrs), follow up grade 9 (15-16yrs) TS 2,3 start TS 4,5 end	3-4 Years Additional time in PE classes Ex: 40min 4 x week 3 of 4 classes: weight bearing activities, jumping, running, gymnastics, ball games 1 of 4 classes: swimming Con: regular PE classes of 60 min 2 x week Compliance: Ex 93%, Con 91%	DXA: BMC, aBMD, vBMD, and bone size: TB, LS, FN SXA: BMC and aBMD: distal radius and ulna QUS: BUA, SOS, and SI: calcareous (heel)	3/4 Years Boys: BMC FN: +8% / 0% aBMD FN: +9% / +14% vBMD FN: 9% / +15% BMC LS: +9% / 0% aBMD LS: 0% / +10% SOS Heel: +1% / +11% SI Heel: +7% / +2% 3-4 Years Girls: aBMD distal/ultra-distal radius: -6-7%	vBMD and BA was derived Con girls had high levels of leisure PA, bone mass, Ca intake and earlier menarche than Ex girls, which may have masked effects of intervention Ex program not specific to building bone Control group not from the same school
Pubertal (Tanner Stage 4-5)					
Blimkie et al. (1996)	Girls Ethnicity not reported Ex: n=16, Con: n=16 Age range: 15.9-16.3 All postmenarcheal	6.5 Months Machine assisted weight training 3 x week 4 sets of 12 reps each, with progression every 6 weeks	DPA BMC: TB and LS aBMD: TB and LS	NS differences in any of the bone variables measured	Compliance was no clear The duration/length of each session was not clear
Heinonen et al. (2000) (Part B)	Girls, White Ex: n=39, Con:, n=29 Age range: 12.8-15yrs Selection to groups decided by teachers	9 Months Step aerobic program: 50 min 2 x week with 20 min of jump exercises: 100-200 jumps from box (two and one footed) Ground rx forces not measured Compliance: Ex 65%, Study 92%	DXA and pQCT BMC: LS and FN Cortical area: tibial midshaft	NS differences in any of the bone variables measured	Compliance low Potential selection bias due to teachers selecting groups

Ex: exercise group; Con: control group; BMC: bone mineral content; aBMD: areal bone mineral density; vBMD: volumetric BMD; BA: bone area; BMAD: bone mineral apparent density (BMD adjusted for BA); TB: total body; LS: lumbar spine; FN: femoral neck; NN: narrow neck; PF: proximal femur;, WT: wards triangle; TR: trochanter; SXA: single energy x-ray absorptiometry; DXA: dual energy x-ray absorptiometry; DPA: dual photon absorptiometry; QCT: qualitative computed tomography, pQCT; peripheral QCT; HSA: hip structural analysis; QUS: quantitative ultrasound; SOS: speed of sound; SM: section modulus; CSMI: cross-sectional moment of inertia; IBS/BSI: index of bone structural strength; SSI: strength strain index; BUA: broadband ultrasound attenuations; Ca: calcium; Rx: reaction; BW: body weight; PE: physical education; TS: Tanner stage; Pl: Placebo; Grps: Groups; NS: no significant.

Table 3.2 Continued - Intervention studies on the effects of exercise on bone indices in youth.

Reference	Population	Intervention	Measures	Results	Limitations
Pubertal (Tanner Stage 4-5)					
Nichols et al. (2001)	Girls Ethnicity not reported Ex: n=5, Con: 11 Age range: 14-17 yrs All postmenarcheal Randomized groups	15 Months Resistance training program weights and machines: 30-45 min, 3 x week of 15 Progression: weight increase Compliance: Ex. 73%, Study 15%	DXA BMC and aBMD: TB, LS, FN, WT, and TR BMAD: LS and FN	aBMD WT: +3.2% aBMD FN: +2.3%	Large dropout rate resulting in small sample size (originally Ex=46, Con=21)
Schneider et al. (2007)	Girls, White, Hispanic, Asian Ex: n=63, Con: n=59 Age range: Randomized two schools: 1 Ex + 1 Con All given 500mg Ca/d	10 Months, 2 school semesters School based program: 60 min 5 x week (~40min activity time) Variety of aerobic (3 x week), strength building (1 x week), educational (1 x week) activities	DXA + bone turnover BMC and BMD: TB, LS, Hip, thoracic spine, FN and TR Bone formation: OC, BSAP, and CICP Bone resorption: PYD	Thoracic BMC: +4.9% NS differences in BMD measurements or markers of bone turnover	Compliance not reported Population may not be generalizable as proactive approach to attrition taken and terminated participation Duration of study time points is unclear
Stear et al. (2003)	Girls, White Total n=144 Age range: 16-18 yrs Ca Ex: n=37 Ca No Ex: n=28 Placebo Ex: n=38 Placebo No Ex: n=28 All postmenarcheal Randomized, double blinded 2 schools	15.5 Months Lunch + after school program 45min 3 x week of aerobic to music: moderate to vigorous high impact movements Ground rx forces not measured Ca: 1000mg/day Ex attendance: 36% Ca compliance: 70%	DXA BMC and BA: TB, LS, FN TR, hip, nondominant total, ultradistal and distal third radius	Ca Ex > Placebo No Ex BMC TB: +0.8% BMC LS: +1.9% BMC FN: +2.2% BMC Hip: +2.7% BMC TR: +4.8% Ex > No Ex BA LS: +0.7% BMC Hip: +1.4% BMC TR: +2.6%	Poor Ex attendance Decreased BA in the hip which may suggest reorientation of the hip with increasing age, redistribution of mineral, or alternation in bone-edge detection of DXA Results based on good compliance (smaller sample)

Ex: exercise group; Con: control group; BMC: bone mineral content; aBMD: areal bone mineral density; vBMD: volumetric BMD; BA: bone area; BMAD: bone mineral apparent density (BMD adjusted for BA); TB: total body; LS: lumbar spine; FN: femoral neck; NN: narrow neck; PF: proximal femur;, WT: wards triangle; TR: trochanter; SXA: single energy x-ray absorptiometry; DXA: dual energy x-ray absorptiometry; DPA: dual photon absorptiometry; QCT: qualitative computed tomography, pQCT; peripheral QCT; HSA: hip structural analysis; QUS: quantitative ultrasound; SOS: speed of sound; SM: section modulus; CSMI: cross-sectional moment of inertia; IBS/BSI: index of bone structural strength; SSI: strength strain index; BUA: broadband ultrasound attenuations; Ca: calcium; Rx: reaction; BW: body weight; PE: physical education; TS: Tanner stage; Pl: Placebo; Grps: Groups; NS: no significant.

Table 3.2 Continued - Intervention studies on the effects of exercise on bone indices in youth.

Reference	Population	Intervention	Measures	Results	Limitations
Pubertal (Tanner Stage 4-5)					
Weeks et al. (2008)	Boys and Girls Total n=81 Ex Boys: n=22 Con Boys: n=15 Ex Girls: n=21 Con Girls: n=23 Age range: 13.5-14.5 Randomized 1 school	8 Months Ex: 10 min 2x week jumping activities as warmup in PE class worked up to ~300 jumps at 1-3 Hz, height 0.2-0.4m Con: 10min 2x week of regular PE class warmup Compliance Ex 80% Study dropout rate 18%	DXA and QUS BMC, BMD, and BA: TB, FN, LS, TR BMAD, CSMI, IBS, and cortical wall thickness BUA: nondominant calcaneus	Boys: BMC TB: +4.3% NS increases Ex boys: BUA calcaneus: +3.6% FN area: +1.1% Girls: NS differences NS increases Ex girls: BMC FN: +9% BMAD LS: +3.7% LS area: +2.9%	Volumetric bone densities were derived/estimated Small sample size for between group sex differences
Witzke & Snow. (2000)	Girls, White Ex: n=25, Con:, n=28 Age range: 14-15 yrs All postmenarcheal No randomization	9 Months Ex: 30-45 min 3 x week of resistance and plyometrics training with increasing intensity over 9 months Ground rx forces not measured	DXA BMC: TB, LS, FN, TR	NS differences in BMC between groups However, increases in BMC for TB, LS, FN, TR ranged +0.1-2.1% in Ex group	Compliance not reported Potential selection bias

Ex: exercise group; Con: control group; BMC: bone mineral content; aBMD: areal bone mineral density; vBMD: volumetric BMD; BA: bone area; BMAD: bone mineral apparent density (BMD adjusted for BA); TB: total body; LS: lumbar spine; FN: femoral neck; NN: narrow neck; PF: proximal femur;, WT: wards triangle; TR: trochanter; SXA: single energy x-ray absorptiometry; DXA: dual energy x-ray absorptiometry; DPA: dual photon absorptiometry; QCT: qualitative computed tomography, pQCT; peripheral QCT; HSA: hip structural analysis; QUS: quantitative ultrasound; SOS: speed of sound; SM: section modulus; CSMI: cross-sectional moment of inertia; IBS/BSI: index of bone structural strength; SSI: strength strain index; BUA: broadband ultrasound attenuations; Ca: calcium; Rx: reaction; BW: body weight; PE: physical education; TS: Tanner stage; Pl: Placebo; Grps: Groups; NS: no significant.

3.3.1 Pre-pubertal Interventions

As shown in Table 3.2, positive effects of exercise on bone indices were found in 13 of 16 studies (81%), with overall effects ranging from 0.6% to 9.5% depending on the skeletal location and the type of measure (BMC, BMD, etc) taken for studies 7-36 months in duration. The average percent improvements for BMC included 4.5%, 4%, 2% and 1.5% at the lumbar spine (LS), femoral neck (FN), femur and total body (TB) respectively. BMD gains across studies were between 0.6-3% for the LS, FN and TB. The largest gains in girls was in BMC and area of the forearm (12.5% and 13.2% respectively) using peripheral DXA after 36 months of increased physical education class time (Hasselstrom et al., 2008). The one study that used pQCT in this group (Macdonald et al., 2007) exhibited the largest bone gains in boys after 16 months of jump training, reporting an increase of approximately 25% in BSI (an index of bone structural strength) of the distal tibia. MacKelvie et al. (2004) also presented large gains using HSA, with boys seeing a 12% increase in FN cross-sectional moment of inertia.

Despite the bone gains being similar between boys and girls, the number of studies that reported significant increases differed (4 vs. 7 out of 8 for girls and boys, respectively). These discrepancies can largely be explained by the differences in the duration and type of intervention employed. MacKelvie et al. (2001) and (2002) utilized 7 months of school-based physical education classes to employ a jump circuit intervention which elicit ground reaction forces 3-5 times one's body weight, and demonstrated favourable gains in bone in boys but not girls. Fuchs et al. (2001) also found 7 months of jump training to be favourable to improvements in both LS and FN BMC and BMD in prepubertal boys and girls. In fact, the gains demonstrated in Fuchs et al. (2001) were greater than those in the MacKelvie et al. (2001, 2001) studies, most likely due to the larger ground reaction forces generated (8.8 vs. 3.5-5 x

body weight). Studies at 12 months (Alwis et al., 2008b; Linden et al., 2007) utilizing a weight-bearing physical education intervention follow a similar trend, with improvements being seen in boys but not girls, which could be related to a higher intensity of the intervention achieved by the boys. The extra intervention time has not helped to elicit a significant positive bone response in the young girls. It is not until 24 months of the same type of weight-bearing activity intervention that positive gains are found in girls (Linden et al., 2006). It would therefore appear that improvements in bone as a result of a physical activity intervention would more likely occur in prepubertal boys than girls. This was particularly obvious after 7 months of jumping training (MacKelvie et al., 2001, 2002) and 12 months of weight-bearing activities (Alwis et al., 2008b; Linden et al., 2007). Improvements in prepubertal girls were seen in studies lasting 24 months in duration (Linden et al. 2006); any studies demonstrating bone gains in a mixed gendered population (Fuchs et al., 2001; McKay et al., 2000) could be due to greater changes in the boys than the girls.

3.3.2 Early Pubertal Interventions

Eighty-three percent of the physical activity interventions were capable of creating a positive effect on bone strength parameters in pubertal boys and girls (Table 2). Study durations ranged from 3 months to 4 years, with both the average and median duration being 12 months. The percent gains in bone ranged from between 1.3-15% and again depended on the measurement location and the technique employed. Of the sixteen studies conducted in this group, 12 utilized DXA, 3 used pQCT, 2 used QUS, and 1 used SXA. Three of the DXA studies also conducted HSA with two of the overall studies employing more than one technique to assess the effects on bone. The largest improvements in bone for girls was a 10.3% change in

aBMD at the FN following 10 months of a mixed program using jumping, weight-bearing exercise and weight training (Morris et al, 1997). In boys, the greatest improvements were in the double digits at 10%, 11%, 14% and 15% for LS aBMD, calcaneal SOS, FN aBMD and vBMD, respectively (Sundberg et al., 2001). These finding in boys were demonstrated after 4 years of increased physical education classes that involved a mixed program of weight-bearing and jumping activities. In addition to a physical activity intervention, two of the studies also employed a calcium intervention (Courteix et al., 2005; Iuliano-Burns et al., 2003). These studies (Courteix et al., 2005; Iuliano-Burns et al., 2003) demonstrated that calcium supplementation in addition to physical activity can elicit greater responses in bone than does exercise alone. This finding highlights the importance of monitoring calcium intake during intervention studies, particularly during puberty.

The number of interventions conducted in boys versus those conducted in girls was not equal as was the case in the prepubertal group, which makes the discussion on sex differences and effects of physical activity on bone in this group problematic. Three studies in early pubertal children by the same author (Macdonald et al., 2007, 2008, 2009) incorporated 16 months of 60 minute weekly classroom physical activity including 5-36 jumps per day, 4 times a week. Measurements with pQCT, DXA and HSA demonstrated no significant changes in bone strength in the tibia, but did show improvements in tibial geometry and bending resistance in boys (Macdonald et al., 2007, 2009). Boys also experienced improvements in lumbar spine BMC and whole body BMC, Girls had increases in section modulus (a measure of bending resistance) of the femoral neck (Macdonald et al., 2008). These results imply that there may be sex differences in the properties of bone that improve following an exercise intervention. The trends shown by Macdonald et al. (2007, 2009), however, failed to reach significance for three

reasons: Firstly, there was an uneven distribution of sample size, maturity status and sex between groups, which made some of the groups underpowered. Secondly, as ground reaction forces were not reported, it is possible that external loads applied during the intervention were not high enough to instigate a loading response in bone. Thirdly and most likely, the benefits of the jumping intervention could have been attenuated due to the low compliance to the program. In fact, Macdonald et al. (2008) reported significant findings for individuals with 80% compliance. Likewise, three studies (MacKelvie et al., 2001, 2003; Petit et al., 2002) demonstrated improvements in BMC, aBMD and vBMD in girls who followed a shorter jumping program (7 months), eliciting larger ground reaction forces (3.5-5 x body weight) and for whom study compliance was 80% (MacKelvie et al., 2001).

It appears not only that larger loading responses are needed to elicit positive changes in bone, but also that the manner in which that load is applied to bone is relevant. A large number of studies (69%) employed specific jumping exercises as part of their intervention and demonstrated that short, irregular, diverse large loads at varying times of the day are sufficient to instigate bone responses (Heinonen et al., 2000; MacKelvie et al., 2001; McKay et al. 2005, Meyer et al., 2011; Petit et al., 2002). Unlike the studies conducted in prepubertal youth, interventions prescribing weight-bearing activities do not need to be conducted over long periods of time to see similar responses in bone. Barbeau et al. (2007), Courteix et al. (2005), and Morris et al. (1997) demonstrated such improvements in 7-12 month's time. Interventions in which there were no improvements in bone parameters attributed this to higher levels of leisure activities in the non-experimental groups, increased bone mass at baseline, and earlier menarcheal status (Petit et al., 2002; Sundberg et al., 2001). All of these factors would

contribute to bone indices being elevated prior to the intervention, which would allow for only small changes to occur and in turn mask any effects of the intervention program.

3.3.3 Pubertal Interventions

The fewest physical activity interventions were conducted in pubertal youth, with all seven involving girls and one also including boys. As shown in Table 3.2, the types of interventions included resistance training (Blimkie et al., 1996; Nichols et al., 2001), jumping trials (Weeks et al., 2008), a combination of resistance and plyometric exercises (Witzke and Snow, 2000), and those with a variety of different weight-bearing activities (Heinonen et al., 2000, Schneider et al., 2007, Stear et al., 2003). DXA was the predominant method used to assess bone in this population, with one study using DPA (Blimkie et al., 1996). Three of the studies that used DXA also used an alternate method such as pQCT (Heinonen et al. 2000), QUS (Weeks et al., 2008) and serum biochemical markers of bone turnover (Schneider et al., 2007). Half of the trials demonstrated significant changes (0.7-4.9%) in bone following their interventions, with three of the studies demonstrating non-significant trends (Schneider et al., 2007; Weeks et al., 2008, Witzke and Snow, 2000). Of those studies that reported significant effects, one included both an exercise and calcium intervention and observed bone mineral advantages at the femoral neck, lumbar spine and total body in adolescent girls receiving both interventions (Stear et al., 2003). The girls receiving just the exercise also had significant changes at the hip, albeit the combination of calcium and exercise generated greater improvements (Stear et al., 2003). Schneider et al. (2007) provided all pubertal girls with 500mg of calcium per day, and unlike Stear et al. (2003) only observed significant changes in thoracic BMC, and non-significant trends of increases in BMD and markers of bone turnover.

It is possible that these results failed to reach significance as the intervention by Schneider et al. (2007) was shorter in duration than Stear et al. (2003), and lasted 10 months vs. 15.5 months. Moreover, Stear et al. (2003) observed the greatest differences between exercising calcium takers and non-exercising, non-calcium consuming controls. As everyone in Schneider et al.'s (2007) study was taking calcium, the room for physical activity induced improvement may have been smaller. Regardless of these discrepancies, one thing that is clear from these two studies and those described in the early pubertal section (Courteix et al., 2005; Iuliano-Burns et al., 2003) is that calcium is important to bone health and its use during physical activity interventions will greatly affect results.

Three investigations of the effects of resistance training on bone mineral accrual in pubertal girls were completed, with only one reporting significant changes in bone indices (Nichols et al., 2001). A major difference between the studies that did not find significant changes (Blimkie et al., 1996; Witzke and Snow, 2000) and the one that did (Nichols et al., 2001) was the duration of the intervention trial. It appears that, with resistance training, a longer trial of approximately 15 months is necessary to demonstrate significant improvements in bone, similarly to the 15.5 months of WBPA in Stear et al. (2003). In addition to resistance training Witzke and Snow (2000) used plyometric training and the utilization of this may have resulted in the strong, yet non-significant trends. This perhaps demonstrates that shorter intervention trials that include ground reaction forces can be efficacious at improving bone. Results from studies examining jumping trials (Heinonen et al., 2000; Weeks et al., 2008) 8-9 months in duration have been ambiguous. Heinonen et al. (2000) failed to measure significant changes in bone; however, Weeks et al. (2008) did observe improved total body BMC in pubertal boys but not girls. Of note, Weeks et al. (2008) did measure large percent changes,

albeit non-significant trends, in many different parameters of bone strength in both boys and girls. These trends could be the result of the greater ground reaction forces used in this study compared to that of Heinonen et al. (2000) and could possibly have reached significance if the duration of the trial were longer and/or with a larger sample size per group. A common theme in all of the studies without significant findings or 'almost' measuring differences is poor compliance. If it were not for the issues with compliance, it is possible that these studies would have found significant results.

3.4 Discussion

3.4.1 The Window of Opportunity for Bone Adaptations

The early pubertal period may be the best time to generate skeletal adaptations to physical activity. Studies conducted in more than one maturity group demonstrated positive bone gains in early pubertal girls with no significant increases in prepubertal (MacKelvie et al., 2001; MacKelvie et al., 2003; Petite et al., 2002) or pubertal (Heinonen et al., 2000) girls. When reviewing all of the intervention studies, the greatest average gains in bone regardless of sex, skeletal location and type of activity used was during the early pubertal years. These results are more definitive in girls, as a larger proportion of intervention studies have been conducted on females across puberty, with the sample of boys decreasing with maturity. Despite this trend, longer duration intervention studies where boys most likely transitioned from pre- to early puberty also demonstrate larger gains in bone than in prepubertal boys alone (MacKelvie et al., 2004). Larger skeletal gains were also observed in interventions trials that supplemented with calcium during early puberty (Courteix et al., 2005; Iuliano-Burns et al.,

2003) compared to those supplementing in prepubertal (Bass et al., 2007) and pubertal (Schneider et al., 2007; Stear et al., 2003) stages. Moreover, the velocity for BMC accrual is highest in early puberty, which means prior to menarche for girls (Bailey et al., 1996, 1997; Cadogan et al., 1998), after which accrual rates decrease with age and plateau in late adolescence upon achieving PBM (Davies et al., 2005). The 'window of opportunity' to impart the largest influences on bone development, therefore, may be during early puberty.

Another important factor as to why very few studies reported changes in pubertal youth is due to how bone is accrued in this maturity group. According to Bailey et al. (1996, 1999) peak velocity of BMC accrual for the whole body occurs approximately 0.7-1 year after peak linear growth around the time of menarche, which corresponds to approximately 12-13 years of age in girls. The pubertal girls in the seven studies reviewed (Table 3.2) were between the ages of 13 and 18, however, which put them after the point of peak BMC velocity accrual and instead at the point at which the velocity of bone accrual actually decreases. The schematic representation of PBM and the rate at which bone mass is accrued over time resembles a dose response curve. It would appear that the pubertal girls in these studies are nearing their PBM, putting them near the plateau of the accrual process, and therefore both the rate and amount of BMC that can be accrued during this time is reduced. As a result, detecting significant changes will be difficult as the total overall bone that can be accrued later in adolescence is much less.

The fact that these percent gains are small and non-significant statistically, then, does not mean that they are also not biologically meaningful. Turner and Robling (2003) demonstrated that a 5.4% and 6.9% gain in aBMD and BMC respectively translated into a 64% and 94% increase in the amount of force and energy a bone could absorb before failure. This

suggests that even small changes in bone mass, which are marginally detectable by DXA, can significantly improve bone strength. A little bone, therefore, goes a long way.

3.4.2 Optimal Physical Activity Interventions for Bone Adaptations

Based on our systematic review of the literature, we can deduce that regular exercise can be an effective way to improve bone density, size, and shape, and consequently, improve the mechanical strength of bone. With the variability in the types of interventions used and how they were employed, there is no clear consensus on exactly how we should prescribe exercise in order to see the greatest returns in terms of bone health. In reviewing the literature, however, it becomes clear that, regardless of pubertal stage, the duration of the trial and the intensity in which the activity was employed appeared to matter. If interventions were short in duration (8-10 months), those that utilized jumping activities with high ground reaction forces received the most positive results (Bass et al. 2007; Fuchs et al., 2001; MacKelvie et al., 2001, 2002; McKay et al., 2005; Petit et al., 2002; Weeks et al., 2008). If weight-bearing activities or resistance training was utilized, longer interventions were required (10-24 months depending on maturity) in order to see significant gains in bone (Alwis et al., 2008a; Courteix et al., 2005; Linden et al., 2006, 2007; Morris et al., 1997; Nichols et al., 2001; Stear et al., 2003; Valdimarsson et al., 2006). In terms of frequency of exercise, Turner and Robling (2003) suggest that it is better to shorten each individual exercise session than to reduce the number of sessions, as jump training has been shown to improve BMC when performed at least 3 time per week but not when reduced to 2 time per week. In fact, gains increase when exercise is performed up to 5 days a week with 2 shorter session in one day; as reflected in the interventions reviewed, with significant gains in bone indices being observed in trials occurring

3-5 times per week. Indeed, the most recent intervention study reviewed (Meyer et al., 2011) demonstrates that a variety of different activities in one intervention at random times of the day can be effective in eliciting bone gains. Physical activity, therefore, is beneficial for bone health; a variety of activities utilizing jump and resistance training as well as weight-bearing activities are some of the most effective ways to elicit an adaptive response in bone. Not only is the variety of activities beneficial for bone but it can also help to alleviate the boredom that accompanies exercise regimens.

3.4.3 Methodological Issues

DXA was the technique most often used in the physical activity intervention trials reviewed, and was used to measure BMC and BMD in various skeletal regions of the body. BMD assessed using DXA is an estimation of 'true' bone density, however, and the areal density that is expressed is affected by bone size. Thus, it is difficult to interpret, evaluate and compare BMD in the growing years when there are considerable changes to the size and shape of bone in children (Bailey et al., 1996; Fulkerson et al., 2004; Gordon et al., 2003; Schöenau et al., 2004). Moreover, aBMD is a surrogate measure for bone strength and although BMC and BMD are related to bone strength, it can be difficult to infer information regarding strength from studies using these measures. This fact is represented in the many studies that cite increases in BMD and BMC that were not always significant. For example, later in puberty when the rate of BMC accrual is decreasing there can be smaller observable, albeit non significant changes, in BMD or BMC compared to earlier pubertal time periods.. Still, these small detectable changes in bone mass using DXA may signify improvements in bone strength, most likely by favourably altering bone geometry (Turner & Robling, 2003). Therefore, the

best parameter for assessing the effectiveness of physical activity interventions on bone would be to use a technique that includes measures of bone mass but also bone shape and size.

pQCT is a method that can be used to detect true vBMD, bone strength, shape and size. Unfortunately, only 5 of the studies that we reviewed utilized this method. An advantage of using pQCT to compare bone structural differences is that it has the capability to demonstrate bone strength adaptations in bone size via changes in cortical thickness or area through investigation of periosteal or endocortical expansion (Haapasalo et al., 2000; Kontulainen et al., 2002; Nikander et al., 2009). To date, however, only one study has measured biochemical markers of bone turnover in response to a physical activity intervention (Schneider et al., 2007). Measuring bone turnover would allow for detection of potential exercise effects sooner, as gains in bone markers have been demonstrated after 8 weeks of resistance training in women 20 years of age (Lester et al., 2009). Further, reference values for many of the markers have been set within the literature, which allows for comparison across studies. Comparisons are difficult to make for static measures of bone as the standards and definitions defining low bone mass are available only for postmenopausal women and not youth.

One way to avoid the above methodological issues is to cease relating bone mass and strength to age, and relate it instead to muscle function (Schöenau and Fricke, 2008). This new methodological concept is based on the thought that the critical property of bone is strength rather than weight and that what influences bone strength are the mechanical loads it must endure either through physical activity or muscle contraction. Regardless of the mode of mechanical load, the stability of the bone must be adapted to muscle strength, which, in a sense, creates a functional muscle-bone unit (Schöenau & Fricke, 2008). Such an analysis removes the concept of a ‘peak bone mass’, which in fact is something we are not capable of

measuring for an individual. Instead, this approach allows for determination and comparison of bone deficits irrespective of age, since bone strength is related to the strength and function of muscle (Schöenau & Fricke, 2008). Moreover, this approach moves away from looking at bone as a separate entity but as functionally linked system.

3.5 Conclusions

With the current growing trends of inactivity and unhealthy dietary habits, the body composition of youth is changing. This new reality makes this systematic review regarding exercise interventions utilizing resistance training versus ground reaction forces relevant. For long-term gains, it appears that short-duration, high-impact exercises undertaken early in childhood (pre and early puberty) have a persistent effect on bone over and beyond that of normal growth and development. Benefits in total body, lumbar spine, thoracic and femoral neck BMC (2.3-4.4%) as well as BMC at the hip (1.4%) have respectively been observed at 3 (Gunter et al., 2008b) and 5 years (Gunter et al., 2008a) following the jumping intervention by Fuchs et al. (2001). It is therefore redundant in some respect to conduct more physical activity interventions unless more advanced techniques of measuring bone are used, as it is apparent from this review that physical activity in a structured, controlled environment is effective in creating positive gains in bone. The next step is to influence change by having schools adopt these activities into their physical education curriculums, or through providing youth with the tools to administer this change on their own.

3.6 Systematic Review Updated

Since our published systematic review of physical activity interventions in children and adolescents included articles until 2011, another search was conducted to determine if any new interventions had been published. The purpose was to update the findings of the systematic review by searching for studies from 2011 to present day (August, 2016). The same eligibility and search criteria were used as outlined in Section 3.2.1. During this time, one narrative (Tan et al., 2014), one systematic review (Nogueira et al., 2014) and four meta-analyses (Behringer et al., 2014; Ishikawa et al., 2013; Nogueira et al., 2014; Specker et al., 2015) have been published on the effects of exercise and physical activity interventions on bone properties in youth. Moreover, the interventions included in the above reviews were derived from literature searches up until 2013 (Dec 2010-January 2013), and provided an additional three studies (Anliker et al., 2012; Bianchini et al., 2013; Lofgren et al., 2012) beyond the 35 included in our systemic review. Our latest search in August 2016 yielded a total of 13 new intervention studies (Bernardoni et al., 2014; Daly et al., 2016; Detter et al., 2013; Fritz et al., 2016a, 2016b; Heidemann et al., 2013; Larsen et al., 2016; Lofgren et al., 2011; Meyer et al., 2013; Seabra et al., 2016). In fact, most of these studies were a follow-up (Meyer et al., 2013) or continuation (Detter et al., 2013; Heidemann et al., 2013; Fritz et al., 2016a, 2016b; Lofgren et al., 2011, 2012) of studies previously discussed in our systematic review (Linden et al., 2006, 2007; Meyer et al., 2011).

Similarly to our systematic review, these reviews and meta-analyses investigated a variety of intervention types, mainly as school-based programs, with bone property outcomes being predominantly DXA derived measures of BMC and aBMD. The distribution of these studies regarding the methodology used to assess bone properties, maturity status, and sex of

participants are similar to what was reported in the systematic review we conducted. The only difference was in the length of some of the newer interventions lasting much longer (up to 7 years) than the ones previously reported. The consensus from these reviews is in agreement with our systematic review that the efficacy of training in terms of bone mineral accrual is substantially affected by maturational status, with WBPAs having significant effects on BMC in pre-pubertal children (Behringer et al., 2014, Specker et al., 2015). However, our systematic review remains the only published review discussing and including non-invasive and dynamic measures of bone, including QUS and biochemical markers of bone metabolism, respectively.

All of the newly reviewed intervention studies found positive improvements in the bone properties, except the one study by Anliker et al. (2012), who did not observe significant improvements in tibial bone strength or geometry after 9 months of jumping exercises (10 minutes, 2 times per week, conducted at start of PE class) despite mirroring a similar protocol by Weeks et al. (2008). The difference between these two studies was the number of jump cycles completed, with Anliker et al. (2012) working up to 150 jumps/session 2 days/wk (300 cycles/wk) by the end of the intervention as opposed to the 300 jumps/session 2 days/wk (600 cycles/wk) in Weeks et al. (2008). It is likely the total 300 jumps/wk did not have a high enough osteogenic index (OI) to stimulate similar positive changes as the 600 jumps/wk. Comparing these two studies using findings by Turner and Robling (2003) (Figure 3.1), we can see a slight increase in the OI index when using 600 versus 300 jump cycles/week (33 vs. 30, respectively) for the same number of loading sessions per week (2 days/wk). According to Turner and Robling (2003), the OI is best improved when adding more loading or exercise sessions per week rather than lengthening the duration (the number of jumps) of each individual session. This is evident in previously reviewed studies (Macdonald et al., 2007,

2008, 2009), which found positive results when incorporating 5-36 jumps per day, 4 times/wk, for a total of 144 jump cycles/wk. Despite there being fewer jump cycles per week than the 300 applied in Anliker et al. (2012), the protocol in the Macdonald et al. (2007, 2008, 2009) studies found positive results due to more but shorter sessions per week having a larger OI (~45), as suggested by Turner and Robling (2003).

Our original systematic review, in combination with the updated literature search, adds to our understanding of the benefits of using jumping protocols to improve bone properties in pre to early pubertal children. Together, these results may speak to a potential jumping threshold below which positive changes in bone properties will not be observed. An OI for walking 20 minutes/day, 5 days/wk, is approximately 36.8 (Turner & Robling, 2003), and the positive results of Weeks et al. (2008) and Macdonald et al. (2007, 2008, 2009), with an OI of 33 versus 45 respectively, are approximately at this level or higher (Figure 3.1). Interestingly, Anliker et al. (2012) with an OI of 30, which is below this level, found no significant improvements in bone properties. In fact, the consensus from the new reviews and meta-analyses support the findings of our systematic review that jumping exercises stimulate bone the best, and suggest jumping as the optimal way to promote positive changes in bone parameters in a short period of time. This is of course providing that the protocols utilized are above the aforementioned OI threshold and conducted with at least 600 cycles/wk separated into 2 loading days/wk (Weeks et al., 2008).

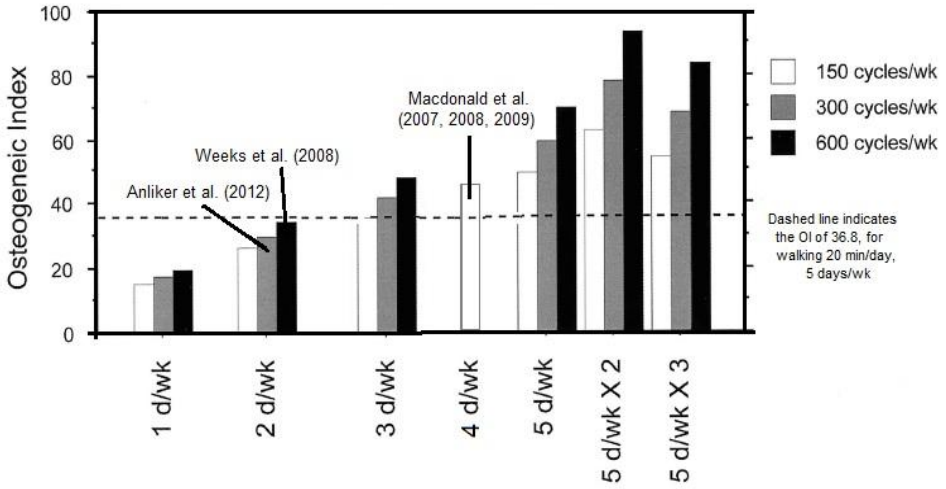

Figure 3.1 Comparison of calculated osteogenic index (OI) from various studies, adapted from Turner and Robling (2003).

Our original review had suggested that for interventions utilizing WBPAs, longer duration is needed to see positive results. This suggestion is reflected in the newer interventions, which showed positive long lasting effects of extended, moderately intense, general exercise interventions (Daly et al., 2016; Detter et al., 2013; Fritz et al., 2016a, 2016b; Lofgren et al., 2011, 2012). The major contribution of the newly found intervention studies comes from their longer durations allowing us to ascertain prolonged effects of exercise, initiated in the pre to early pubertal years, on bone properties. The follow-up study by Meyer et al. (2013) demonstrated the effectiveness of a 9-month general physical education program including jumping exercises to retain positive benefits on BMD and BMC that persisted 3-years after the intervention ended. These results are similar to the retained benefits from an earlier jumping intervention by Fuchs et al. (2001) and 3-5 years later by Gunter et al. (2008a, 2008b). The positive benefits that persist after the cessation of these interventions has been attributed to the maintained current habitual physical activity of former participants (Meyer et al., 2013).

What is still unclear is whether bone enhancing activities performed during childhood and adolescence influence bone strength in older adults, when fracture risk is greatest, and whether past, present, or persistent physical activity is more important for long term bone health. The Malmo Pediatric Osteoporosis Prevention (POP) study is a prospective controlled physical activity intervention study designed to examine the effects of increased school-based physical education on bone health outcomes in Swedish children. This study examined the long-term benefits of an exercise intervention 3 years (Lofgren et al., 2011), 4 years (Lofgren et al., 2012), 5 years (Detter et al., 2013) and 7 years (Fritz et al., 2016a, 2016b) after its initiation. In addition, the POP study estimated annual fracture incidence rate ratios (IRR), and despite a decrease in the IRR with each year of the intervention, there was no significant reduction in fracture risk after 3-5 years of continuous exercise (Detter et al., 2013; Lofgren et al., 2011, 2012). It took 7 years of continuous exercise to significantly reduce fracture risk by decreasing the IRR by almost 50%, which was largely attributed to increases in various bone properties and muscle strength (Fritz et al., 2016a). This finding has very practical applications to bone development and osteoporosis prevention, as it appears that persistent physical activity is important to maintain favourable changes in bone properties over time and in turn fracture prevention (Detter et al., 2013; Lofgren et al., 2011, 2012; Fritz et al., 2016a, 2016b; Meyer et al., 2013).

Physical activity is one of the most efficacious ways for increasing bone strength during the growing years (Heaney et al., 2000); however, children and adolescents are currently not active enough to optimize the health benefits of physical activity (Troiano et al., 2008). This is particularly troubling for girls, as physical activity levels decline greatly during adolescence at a time when bone accrual is at its greatest, and is most sensitive to the potential osteogenic

effects of physical activity (Baptista and Janz, 2012). Some of the newly reviewed studies (Detter et al., 2013; Fritz et al., 2016b; Lofgren et al., 2012) demonstrated greater post-intervention gains in bone properties of girls compared to boys, making maintained physical activity throughout growth, particularly leisure/habitual activity, important to long term bone health and fracture prevention. Boys tended to experience smaller gains likely due to their greater leisure activity levels beyond the intervention program.

More importantly, as related to this dissertation, Tan et al. (2014) was the only review to consider and describe the role that muscle plays on the bone response to loading and to single out the few studies, intervention (Macdonald et al., 2007, 2008) or observational (Burt et al., 2012; Erlandson et al., 2001; Faulkner et al., 2003; Forwood et al., 2006; Greene et al., 2005; Janz et al., 2003, 2007), that discerned the specific contribution of muscle function (or its surrogates) to bone strength. Considering muscle size, strength or LBM is particularly important when investigating the effects of physical activity needed to improve bone strength because muscle has the potential to mediate the relationship between PA and bone (Tan et al., 2014). Once a measure of muscle was added to the few observational studies reported by Tan et al. (2014), the independent role of physical activity diminished illustrating the specific mechanical influence of muscle on bone strength (Burt et al., 2012; Erlandson et al., 2011; Faulkner et al., 2003; Forwood et al., 2006; Greene et al., 2005; Janz et al., 2003, 2007). Moreover, muscle strength has been shown to be a strong and consistent predictor of bone strength (Lorbergs et al., 2011; MacDonald et al., 2008; Schoenau et al., 2002). Some of the newer interventions measured muscle function or size separately but did not take it one step further and relate it back to its potential influence on bone (Anliker et al., 2012; Fritz et al., 2016a). These results highlight the need for studies to take into account muscle function more

often and treat the development of bone as a system connected to muscle, thus as a muscle-bone unit.

In closing, at the end of our systematic review we discuss that the mechanical loads experienced by bone come through either physical activity or muscle contraction, and that studies need to begin relating bone strength to muscle function, a functional muscle-bone unit. This sentiment is shared by Tan et al.'s (2014) narrative that expressed the need to separate the loading influences of physical activity and muscle function. This narrative motivated us to conduct two observational studies that investigated the influence of muscle properties on bone strength apart from weight-bearing or ground reaction forces. Tan et al. (2014) also emphasized the importance of the independent role of muscle on bone and its association with sex, maturation and physical activity be considered in studies with children and adolescents in order to provide a comprehensive picture of mechanisms that drive bone adaptations. These are the factors, in addition to nutrition and bone metabolism, that we attempted to investigate in Part 2 of this dissertation. Taken together, this updated systematic review and recommendations of Tan et al. (2014), underscore the theoretical framework (see Figure 1.1) we used to conduct the cross-sectional and longitudinal studies in the second part of this dissertation.

CHAPTER 4

Mechanical, Biochemical and Nutritional Determinants of Bone Properties in Boys and Girls[2]

4.1 Introduction

To date, the majority of the research on bone growth and development in youth has focused on increasing bone mineral accretion in reference to peak bone mass in order to reduce the risk of osteoporosis-related fractures later in life (Baxter-Jones et al., 2003, 2008; Cadogan et al., 1997; Valimaki et al., 1994; Wang et al., 2007). However, it has been suggested that the critical property for bone health is bone strength rather than bone mass, and that the development of bone strength occurs through appropriately applied mechanical loads placed on bone, e.g., muscle contractions (Rauch et al., 2004; Schöenau & Frost, 2002). In particular, the concept of a muscle-bone unit challenges the notion that bone mass should be related to age, but rather, and perhaps more importantly that bone strength is a function of muscle strength (Schöenau & Fricke, 2008). Other factors known to affect bone, such as physical (in)activity and nutrition, can help or hinder the muscle-bone unit relationship, with bone metabolism regulating the balance between bone strength and deformation (Schöenau, 2005a, 2005b). Examining bone strength from this perspective provides a functional model of bone

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development that is based on the *Mechanostat Theory*, which suggests that increasing the force applied on the bones leads to increasing bone adaptation (Rauch & Schöenau, 2001). During growth, some of the largest loads are applied by muscle contractions. Cross-sectional studies have demonstrated positive relationships between bone properties and muscle mass or force in youth (Janz et al., 2015; Macdonald et al., 2006; Schöenau et al., 2000, 2002; Wang et al., 2007; Wey et al., 2011) without necessarily taking into consideration factors such as maturity and bone resorption.

Furthermore, studies that examined the muscle-bone unit relationship often used surrogate measures of muscle strength (e.g., muscle cross-sectional area) or bone strength (e.g., bone mineral density or content, bone areas). However, these measures are confounded by muscle or bone size. Studies using other techniques to measure bone or muscle strength, such as peripheral quantitative computed tomography (pQCT) or grip force respectively, are scarce (Okumus et al., 2006; Schöenau et al., 1996; 2002; Tenbrock et al., 2000). Grip strength (in relation to radial bone) has predominantly been evaluated in clinical pediatric populations (Okumus et al., 2006; Tenbrock et al., 2000) or adults (Frank et al., 2010; Hasewega et al., 2001; Lorbergs et al., 2011). A few studies conducted in normal healthy children have examined associations between grip strength and whole body BMC (Gracia-Marco et al., 2011; Vicente-Rodríguez et al., 2008), upper arm BMC (Gracia-Marco et al., 2011) and calcaneal bone properties (Herrmann et al., 2015). Furthermore, many of these studies fail to include other modulating factors that contribute to the functional model of bone development (Rauch & Schöenau, 2001). Such systemic factors including hormones, biochemical markers, physical activity and diet can modulate the muscle-bone unit relationship by having both direct and indirect effects on muscle and bone that are not mutually exclusive (4.1). Previous studies have

shown that appropriate daily calcium intake is important to enhancing bone mineral acquisition in girls (Cadogan et al., 1997; Valimaki et al., 1994) while habitual physical activity has been shown to enhance both lean mass and bone accrual in youth (Baxter-Jones et al., 2003; Baxter-Jones et al., 2008).

To appropriately assess the functional development of the muscle-bone unit, measures of muscle strength relative to bone strength are needed, as well as measures of potential modulators. Transaxial quantitative ultrasound (QUS) measures the speed of sound (SOS) *along* the bone and so it is not affected by bone size (Njeh et al., 1999). This allows for better comparisons between children of different sizes (Baroncelli, 2008; Foldes et al., 1995). Therefore, the purpose of this study was to investigate the tenets of the functional model of bone development (Figure 4.1), as adapted from Rauch and Schöenau (2001), by examining the relationship between muscle characteristics (size and strength) and non-weight bearing bone properties, as reflected by the SOS at the radius, in peri-pubertal boys and girls. The radius was specifically chosen to separate the influence of muscle properties on bone strength from those of weight-bearing or ground reaction forces (Greene et al., 2005; Macdonald et al., 2006). Based on the functional model as illustrated in Figure 4.1, biochemical components of bone, physical activity and nutritional factors were also examined to determine their systemic influence on the muscle-bone relationship. The purpose of this study was to identify the relative contribution of various factors to the muscle-bone unit relationship.

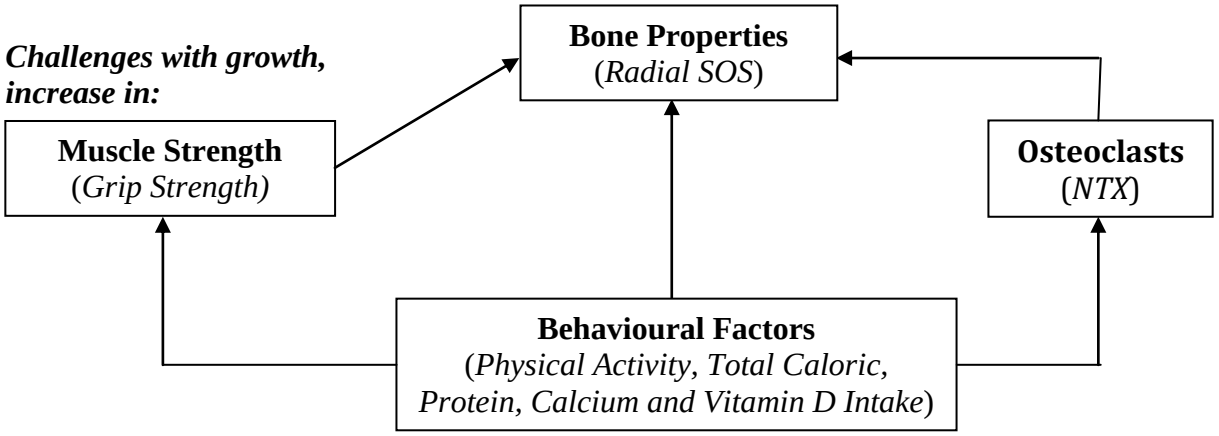

Figure 4.1 Adapted model of functional bone development (based on the model by Rauch & Schöenau, 2001).

4.2 Methods

4.2.1 Participants

A total of 172 children and adolescents (92 boys and 80 girls), 8-16 years of age, were recruited from local school boards in the Niagara Region as well as through poster and information sessions at the University's recreation facility. Individuals with experiences affecting bone properties (i.e., use of steroid medication, growth delay, previous and/or current fracture) were excluded from the study. Girls with irregular menses or using oral contraceptives were also excluded.

4.2.2 Anthropometry and Maturity

Standing and sitting height were measured using a stadiometer (Ellard Instrumentation, Monroe, WA, USA) mounted to the wall and recorded to the nearest 0.1cm. Leg length was calculated by subtracting seated height (height minus sitting height) from standing height.

Body mass was measured to the nearest 0.1 kg using a calibrated balance beam scale (Zenith Digital Scale). Skinfold thickness was measured in triplicate at two sites (triceps and subscapular) using Harpenden calipers (British Indicators, Herts, England), and the median was recorded. If the range in the triplicate measures was greater than 1mm, a fourth measurement was taken and the mean of the two median variables was recorded. Adiposity (percent body fat) was estimated from the sum of these skinfolds, using age- and maturity-specific equations, as described by Slaughter et al. (1988). All measurements were performed by the same investigator to avoid inter-observer variability (ICC=0.996).

The somatic maturity offset (years from age of peak height velocity) was estimated using sex specific regression equations (Mirwald et al., 2002). Age of peak height velocity (PHV) is one of the most commonly used methods of assessing somatic maturity in adolescents (Malina et al., 2004; Mirwald et al., 2002). However, determination of the age of PHV requires serial measurements of growth over a number of years to ascertain peak height velocity, and thus, can only be determined retrospectively from longitudinal data. Mirwald et al. (2002) developed a simple, non-invasive method of assessing somatic maturity in children using known differential growth rate in height, sitting height and leg length. The changing ratio of leg length to sitting height was used to create a sex-specific equation to estimate maturity offset as the years from the age of PHV, thus allowing for the assessment of somatic maturity using a single measurement, rather than serial measurements (Mirwald et al., 2002). Female participants were also asked to indicate their menstrual status including age at menarche (if reached) and frequency of menses in days (see exclusion criteria above). These variables were used for inclusion purposes but were not used in the analysis.

4.2.3 Muscle Size and Strength

Cross-sectional area (CSA, muscle plus bone) was estimated at 65% of dominant forearm length using a simplified anthropometric method (Marganato et al., 1994). Forearm length was measured to the nearest 0.1 cm as the distance between the ulnar styloid process and the olecranon using an anthropometric tape measure. The circumference at 65% forearm length represents the maximal circumference of the forearm and was used to derive the radius with $R1=C/2\pi$. The radius of the limb (muscle and bone) was then calculated from the difference of R1-R2, where R2 is the mean of the forearm anterior and posterior skinfold thickness. This newly calculated radius was then used to calculate muscle-plus-bone using the standard formula of πr^2.

Maximal dominant forearm strength was assessed by a hand-held dynamometer to determine maximal isometric grip force. The device handle was adjusted to the participant's grip size and the test performed with the participant in a standing position with their dominant arm abducted at 45 degrees, with the elbow extended (Lorbergs et al., 2011). Participants were instructed to squeeze the instrument as hard as possible for three seconds. Measurements were recorded to the nearest 0.5 kg. The participant's largest value (best trial) was used to represent their maximal isometric grip force.

4.2.4 Bone Properties and Resorption

Transaxial quantitative ultrasound (Sunlight Omnisense™ 7000S, Sunlight Medical, Israel) was used to assess bone properties by measuring the SOS (m/s) along the bone at the distal 1/3 of the radius. The area of measurement for the radius was the midpoint between the olecranon process and the tip of the third phalanx. Wide scans of 140 degrees around the radius

were performed. All measurements consisted of at least three consistent cycles. A system quality verification of the QUS was performed with a Perspex phantom before the first test of each day. The intra-operator coefficient of variation of the QUS measurements in 10 children was 21% and the interclass correlation coefficient was 0.98. The QUS method measures the SOS *along* the bone and so it is not affected by bone size (Njeh et al., 1999). SOS has also been shown to predict fractures independent of BMD, suggesting that it is measuring an aspect of bone strength (Nguyen et al., 2004). Indeed, QUS measurements are related to bone density, elasticity and microstructure (Gluer et al., 1994), but not to cortical thickness (Njeh et al., 1999). The QUS method is relatively inexpensive, portable, and does not involve any radiation. Thus, it has been recommended for assessment of bone properties in children (Baroncelli, 2008), and has been used to demonstrate the effect of exercise training or physical activity on various bones (e.g., tibia) and in different age groups including children (Daly et al., 1997; Falk et al., 2003, 2007, 2010; Holmes et al., 2010).

Cross-linked N-telopeptides of bone type I collagen (NTX) was measured from first morning mid-stream urine samples and analyzed using enzyme-linked immunosorbent assay (ELISA) kits (Osteomark® NTX Urine Assay, Alere Scarborough, Inc., USA). All samples were analyzed in duplicate with the mean of the duplicate absorbancies used to determine NTX concentrations. All assayed plates were read using the same microplate reader and absorbencies analyzed using GraphPad Prism (GraphPad Software, Inc., USA). Urinary creatinine was analyzed in duplicate using a creatinine colormetric assay kit (MicroVue™, Quidel Corporation, SanDiego, CA, USA) based on a modified Jaffe method. NTX values were corrected for urinary creatinine and final results reported as nmol BCE/mmol creatinine. The intra-assay and inter-assay coefficient of variation for NTX was, 2.5% and 11.6%, respectively.

4.2.5 Physical Activity and Dietary Intake

Physical activity was assessed using Actigraph GT1M accelerometers. The accelerometers were programmed to record activity counts at 5-second epochs along the vertical axis to measure accelerations from 0.05-2 G's at a frequency of 0.25-2.5 Hz. Participants were instructed to wear the belted accelerometer for 7 consecutive days, against the skin, on the right hip for all waking hours. Physical activity logs were used to record times the accelerometer was removed (i.e., during bathing, swimming, competition). Although seven days of monitoring was expected of the subjects, a minimum of three week days and one weekend day, with a minimum of 10 hours of waking data recorded, were used for analysis (Penpraze et al., 2006). A Visual Basic data reduction program was used to quantify total time spent performing moderate, vigorous, and very vigorous physical activity (MVV) according to age-appropriate cut-off thresholds (Trost et al., 2001).

Dietary intake was evaluated using a 24-hour recall interview as previously described (Moore et al., 2007). The 24-hour recall method is the most commonly used assessment tool in large cross-sectional surveys and skeletal development studies in both children and adults (Moore et al., 2007). This method has numerous advantages including responsiveness to change in food supply and habit (Guenther et al, 1997; Harrison et al, 2000).

In brief, participants were asked to recall everything consumed (including foods, beverages, sauces and condiments) the day prior to the interview. Prior to answering the 24-hour dietary recall, participants were asked if the last 24 hours were typical for their diet. If it were not a *typical* day (e.g., birthday party, family gathering, eating out), they reported two days prior to the interview date. The recall started from the first meal or beverage consumed at waking until midnight of the reporting day. Pictures representing different portion sizes of

foods, sizes and measurements of various kitchenware models were used to help ascertain the most accurate amount of food that was consumed. Dietary analysis was conducted (using Nutritionist Pro™, Axxya Systems, USA) to estimate total caloric (kcal), protein (g), calcium (mg) and vitamin D (µg) intake.

4.2.6 Statistical Analysis

Sex-specific descriptive statistics were determined for all study variables by calculating the mean and standard deviation (SD). Skewness and kurtosis were used to assess the assumption of normality for each variable and all were found to be within an acceptable range. Independent sample t-tests were performed to determine differences in participant characteristics, muscle-bone unit and modulator (physical activity and nutrition) variables between boys and girls. Pearson correlations were used to identify relationships between radial SOS, muscle strength and size, MVV physical activity, nutritional intake and NTX. The strength of the relationship noted in bivariate correlations was used to help select predictor variables for the regression models. Hierarchical regression was used to evaluate the contribution of muscle and behavioural variables to dominant radial SOS, with variables entered in blocks in the following order: (1) sex and maturity offset, (2) grip strength, (3) physical activity and dietary calcium, and (4) NTX. Due to the collinearity between age and maturity offset these two variables were examined in the regression analysis separately. In order to be in line with previous studies (e.g. Macdonald et al., 2006), we report the regression results using maturity offset only. However, when the analysis was done with age instead of maturity offset the results were very similar.

Variables were entered in this particular order to help reveal the most appropriate predictors of bone strength from our adapted functional model of bone development (Figure 4.1) based on Rauch and Schöenau (2001). Furthermore, variables were entered in these blocks to see if our results using QUS supported findings previously reported in the literature, in which DXA was used and whether the addition of NTX helped to further explain the functional model of bone development. For this reason, models were run with and without NTX being entered as a predictor variable. Recognizing that predictors may be different between girls and boys, separate regressions for boys and girls were also conducted. Statistical analyses were performed using IBM SPSS Statistics version 21 for Windows (IBM Corp., Armonk, NY). A two-sided alpha level of 0.05 was the criterion for significance for all statistical analyses.

4.3 Results

Values for physical characteristics for boys and girls appear in 4.1, with muscle-bone unit and modulator variables presented in Table 4.2. Boys and girls were similar in age, height, weight, and lean body mass. Girls were more mature than boys and had higher percent body fat ($p<0.001$).

There were no statistical differences between boys and girls in radial SOS or bone resorption (NTX). Although girls appear to be less active than boys, this difference in MVV activity was not statistically significant ($p=0.07$). Boys had greater muscle size and absolute grip strength than girls ($p<0.05$) but these differences in grip strength disappeared when adjusted for muscle-and-bone size (i.e., CSA). There were sex differences in dietary intake, with boys having a greater total energy, protein and calcium intake than girls ($p<0.05$). After correcting for body mass, however, only total caloric intake ($p=0.03$) was higher in boys.

Table 4.1 Participant Characteristics.

	Boys (n=92)	**Girls (n=80)**	**Total Group (n=172)**
Age (yrs)	11.7 ± 2.1	11.7 ± 1.9	11.7 ± 2.0
Maturity Offset (yrs)	-1.7 ± 1.7	-0.2 ± 1.6*	-1.0 ± 1.8
Height (cm)	151.8 ± 14.8	150.4 ± 12.4	151.2 ± 13.8
Body Mass (kg)	46.0 ± 16.0	45.5 ± 13.4	45.8 ± 14.8
Body Fat (%)	17.5 ± 8.5	22.1 ± 7.6*	19.6 ± 8.4
Lean Body Mass (kg)	37.2 ± 10.7	34.8 ± 7.7	36.1 ± 9.5

*Significant sex differences ($p<0.05$); values represented as mean ± SD.

We observed positive correlations between radial SOS and maturity offset, muscle strength and muscle size ($r=0.22$-0.36, $p<0.01$). Grip strength was found to have a slightly greater positive association with radial SOS than forearm muscle size, even after correcting for muscle CSA ($r=027$-0.30 vs. 0.22, $p<0.01$). Radial SOS was also found to be significantly associated with size-adjusted grip strength in girls and with NTX in boys. Radial SOS was negatively correlated with physical activity and NTX ($r=-0.26$ and -0.29, respectively, $p<0.05$). There were no significant correlations between radial SOS and dietary intake. However, all dietary variables including total caloric, protein, vitamin D and calcium intake relative to body mass were associated with NTX ($r=0.21$-0.23, $p<0.01$).

Table 4.2 Muscle-bone unit variables and modulators in peri-pubertal boys and girls.

	Boys (n=92)	**Girls (n=80)**	**Total Group (n=172)**
Radial Speed of Sound (m/s)	3798 ± 88	3815 ± 94	3806 ± 90
NTX (nM BCE/mM creatinine)	547 ± 231	516 ± 287	532 ± 259
Forearm CSA (cm^2)	31.6 ± 8.3	29.1 ± 6.1*	30.4 ± 7.4
Grip Strength (kg)	23.9 ± 7.9	21.3 ± 5.9*	22.7 ± 7.1
Grip Strength/CSA (kg/ cm^2)	0.75 ± 0.13	0.73 ± 0.12	0.74 ± 0.13
Total MVV Activity (min/day)	114 ± 43	101 ± 41	108 ± 42
Total Energy Intake (kcal/day)	1696 ± 500	1492 ± 354*	1589 ± 421
Total Protein Intake (g/day)	69.5 ± 23.5	62.0 ± 18.0*	66.0 ± 21.4
Total Calcium Intake (mg/day)	1015 ± 442	883 ± 383*	947 ± 420
Total Vitamin D Intake (µg/day)	4.9 ± 3.8	4.2 ± 2.4	4.6 ± 3.3
Relative Energy Intake (kcal/kg/d)	40.3 ± 15.2	35.5 ± 13.4*	37.8 ± 14.8
Relative Protein Intake (g/kg/d)	1.63 ± 0.69	1.48 ± 0.61	1.56 ± 0.65
Relative Calcium (mg/kg/d)	24.4 ± 13.7	21.6 ± 12.8	23.0 ± 13.4
Relative Vitamin D (µg/kg/d)	0.12 ± 0.10	0.10 ± 0.07	0.11 ± 0.09

*Significant sex differences ($p<0.05$); values represented as mean ± SD; NTX=Cross-linked N-telopeptides of bone type I collagen; CSA=Cross sectional area; MVV=moderate, vigorous and very vigorous physical activity

Absolute grip strength did not enter as a significant predictor of radial SOS in the regression models. However, in model 2, relative grip strength was the second most important predictor of radial SOS after maturity offset, and alone could explain an additional 4% of the variance in radial SOS over and beyond the 12% explained by the maturity offset and sex in model 1. In addition, once grip strength was entered into the model, sex's contribution in the model was no longer significant (Table 4.3). MVV physical activity was not a significant predictor of radial SOS. In the final model, maturity offset, relative grip strength, dietary

calcium and NTX were found to be the only significant predictors of radial SOS accounting for 21% of the variability in radial SOS (Table 4.3).

Table 4.3 Regression models predicting radial speed of sound (SOS) using maturity, sex, relative grip strength, physical activity, daily calcium intake and NTX.

	Variables	**Model 1**	**Model 2**	**Model 3**	**Model 4**
B1	Maturity Offset (yrs)	20.5 (0.41)**	16.8 (0.33)**	15.8 (31.2)**	14.2 (0.28)*
	Sex	-33.5 (-0.19)*	-25.5 (-0.14)	-25.3 (-0.14)	-25.0 (-0.14)
B2	Grip Strength/CSA		161.1 (0.22)**	140.0 (0.19)*	134.9 (0.18)*
B3	MVV physical activity (min/day)			-0.30 (-0.14)	-0.30 (-0.14)
	Calcium Intake (mg/kg)			1.0 (0.15)	1.2 (0.17)*
B4	NTX (nM BCE/mM creatinine)				-0.1 (-0.20)*
	Adjusted R^2	0.12**	0.16**	0.18**	0.21**

**$p<0.01$, *$p<0.05$; Unstandardized ß-coefficients are reported with Betas in parentheses; CSA=Cross sectional area; MVV=moderate, vigorous and very vigorous physical activity; NTX=Cross-linked N-telopeptides of bone type I collagen;

According to the sex specific regressions, maturity offset, relative grip strength and NTX together accounted for 27% of the variance in radial SOS in boys with NTX being the strongest predictor. In girls, maturity offset was consistently the most significant predictor of radial SOS, alone explaining 19% of the observed variance (Table 4.4). In addition to maturity offset, dietary calcium intake was the only other significant predictive variable of radial SOS in girls.

Table 4.4 Regression models predicting radial speed of sound (SOS) using maturity, sex, relative grip strength, physical activity, daily calcium intake and NTX in boys and girls.

	Variables entered	Model 1	Model 2	Model 3	Model 4
	Boys				
B1	Maturity Offset (yrs)	15.3 (0.29)*	13.2 (0.25)*	6.9 (0.13)*	1.5 (0.03)*
B2	Grip Strength/CSA		132.7 (0.19)*	124.4 (0.18)*	163.0 (0.23)*
B3	MVV physical activity (min/day)			-0.4 (-0.19)	-0.5 (-0.25)
	Daily Calcium Intake (mg/kg)			0.01 (0.06)	0.03 (0.15)
B4	NTX (nM BCE/mM creatinine)				-0.2 (-0.44)**
	Adjusted R^2	0.07*	0.09*	0.09*	0.27**
	Girls				
B1	Maturity Offset (yrs)	26.3 (0.45)**	20.6 (0.35)**	19.8 (0.34)*	20.0 (-0.34)*
B2	Grip Strength/CSA		186.0 (0.23)	169.2 (0.21)	172.2 (0.21)
B3	MVV physical activity (min/day)			-0.15 (-0.06)	-0.15 (-0.07)
	Daily Calcium Intake (mg/kg)			0.06 (0.24)*	0.06 (0.24)*
B4	NTX (nM BCE/mM creatinine)				0.01 (0.02)
	Adjusted R^2	0.19**	0.22**	0.26**	0.25**

**$p<0.01$, *$p<0.05$; Unstandardized ß-coefficients are reported with Betas in parentheses. CSA=Cross sectional area; MVV=moderate, vigorous and very vigorous physical activity; NTX=Cross-linked N-telopeptides of bone type I collagen;

4.4 Discussion

The main purpose of this study was to investigate the functional model of bone development proposed by Rauch and Schöenau (2001) by examining the primary mechanical challenges (i.e., muscle force) to bone strength during growth, along with behavioural factors (i.e., physical activity and nutrition). This was the first study to include an indicator of bone resorption in the investigation of the muscle-bone unit, in addition to combining non-invasive

measures of strength for both muscle and bone. Maturity, relative grip strength, dietary calcium intake and NTX were able to account for 21% of the variance in radial SOS in children 8-16 years of age, with some differences between sexes. In girls, maturity was the strongest predictor of radial bone strength, followed by calcium intake. However, in boys, according to the ß-coefficients the primary explanatory variable was NTX followed by muscle strength and then maturity.

Overall, we have shown that forearm muscle strength is related to radial bone strength, independent of size, even after controlling for sex and maturity. Furthermore, the addition of bone resportion (NTX) to our model contributed significantly to the observed variance in radial bone strength, which helped to further explain the functional model of bone development. Calcium intake only became a significant predictor of radial SOS after adding NTX to the regression model, which suggests that the effect of calcium intake on the muscle-bone unit was modulated through bone resorption. Likewise, all dietary variables including total caloric, protein and vitamin D intake were correlated with NTX.

4.4.1 Mechanical Challenges

Relative grip strength was found to account for an additional 4% in radial SOS over and beyond maturity and sex. In fact, with relative grip strength in the model sex was no longer a significant predictor, suggesting that the effects of sex may be masked by differences in relative grip strength. Normalizing for muscle size may remove the influence of sex by removing the sex-dependent maturity effects on muscle size. This is consistent with a functional model of bone development which postulates that the primary mechanical challenges to bone's *mechanostat* during growth stems from increases in bone length and muscle force (Frost, 1987;

Rauch & Schöenau, 2001). However, the amount of variance in radial SOS explained by our model (21%) was still less than what is typically reported in the literature. In adults, Frank and colleagues (2010) demonstrated that isokinetic grip force, muscle CSA, arm length, and sex explained 79-80% of the variance in radial SSI. Furthermore, Lorbergs and colleagues (2011) found that grip strength alone accounted for 21% of pQCT-derived torsional strength (SSI) at the proximal radius in men and 23% of compression strength (BSI) at the distal radius, in women. In children and young adults, aged 6-22 years, muscle size, as a surrogate for strength, accounted for 77% of cortical area at the proximal radius (Schöenau et al., 2000). Our significant positive associations between muscle force, muscle size and radial SOS are also comparatively weaker than those in the pediatric literature between various bone and muscle measurements at the radius (r=0.22-30 vs. r=0.85-95, respectively) (Schöenau, 2005; Schöenau et al., 1996; 2001; 2002; Tenbrock et al., 2000; Wey et al., 2011), humerus (r=0.54-0.81) (Daly et al., 2004; Wang et al., 2007), and at the tibia (r=0.54-77) (Janz et al., 2015; Macdonald et al., 2006). This discrepancy in the strength of the relationships may be due to differences in bone outcomes (SOS vs. BMD, SSI or BSI), or differences in the site of bone assessment (e.g., distal radius, which is trabecular bone vs. mid-radius, which is cortical bone). It is also possible that the relationship is stronger when using DXA measurements because of an added effect of size. By using QUS measurements and expressing muscle strength relative to muscle CSA we have factored out the effect of size in the relationship, which can explain the lower percent contribution of relative grip strength to the variance of radial SOS.

The results from two studies that have used grip strength to assess muscular strength in healthy children have been able to demonstrate the established relevance of muscle strength being one of the strongest fitness variables correlated with BMC (Gracia-Marco et al., 2011;

Herrmann et al., 2015). Gracia-Marco et al. (2011) demonstrated that lower handgrip test performance was related to decreased BMC in the whole body, upper and lower limbs. In school children, ages 6-10 years, Herrmann et al. (2015) observed that fat free mass (FFM) and muscle strength were positively associated with calcaneal bone strength measured using QUS. Specifically, an additional 1 kg of FFM or handgrip strength corresponded to a 0.5 unit and 0.2 unit increase in calcaneal bone strength, respectively (Herrmann et al., 2015). Similarly, Vicente-Rodriguez et al. (2008) found lean mass to have an independent relationship with bone mass, explaining 67% of the total variance in whole body BMC, with grip strength acting as an independent predictor of whole body BMC. Together, these results of these studies demonstrate that physical fitness-related variables related to strength, such as grip strength, may have predictive value for bone mass and its accumulation during growth. Moreover, the independent relationship between physical fitness, or physical activity, and bone may be mediated by lean mass, as the association between fitness measures and whole body BMC disappeared after controlling for lean mass (Vicente-Rodriguez et al., 2008). This is in contrast to our study, which demonstrated grip strength to remain as a predictor of radial SOS even after controlling for muscle size.

Most studies fail to make direct assessments of muscle strength and instead use surrogate measures such as muscle CSA. This approach is based on the fact that muscle strength generally scales with muscle size and assumes appropriate measures of size are sufficient for muscle-bone unit investigations (Petit et al., 2005). A number of studies have demonstrated muscle strength and power to be similarly predictive of bone strength as muscle size (Frank et al., 2010; Lorbergs et al., 2011; Macdonald et al., 2006), which is in contrast to our findings that grip strength was a stronger determinant of radial SOS than muscle CSA.

Beyond muscle CSA, neuromuscular properties may help to account for increases in muscle strength and function with growth and maturation (Blimkie, 1989), and could explain why relative grip strength and not absolute grip strength was a significant predictor of radial SOS in our regression models.

4.4.2 Bone Resorption

The central piece of bone regulation in the functional model of bone development is the regulatory feedback loop between bone deformation (tissue strain) and bone strength, which is controlled by osteoblasts and osteoclasts (Rauch and Schöenau, 2001). The majority of studies use imaging technologies to examine bone geometry and cite changes in periosteal or endocortical bone surfaces to infer the actions of bone cells (Daly et al., 2004; Macdonald et al., 2006; Schöenau et al., 2000). This is the first study to measure a marker of bone resorption within the context of the functional model of bone development in healthy children. When NTX was included in the regression analysis, we found that it helped to strengthen the predictive model of radial bone strength by accounting for an additional 3% of variance in radial SOS. Future studies should measure markers of both bone formation and resorption to elucidate their contributions to the muscle-bone unit.

4.4.3 Physical Activity and Nutrition

The contribution of physical activity in the regression models for radial SOS was not significant. The influence of physical activity on the muscle-bone unit relationship is ambiguous within the literature and depends on how physical activity is measured, and the type, location, and indices of bone being investigated. We measured MVV physical activity

using accelerometers along the vertical axis, which made our physical activity measures sensitive to weight-bearing types of activity. Since the radius is a non-weight bearing bone, it is not unreasonable that physical activity did not enter as a significant predictor of radial SOS in our model.

Adequate nutrition is important for both muscle and bone development. Total dietary protein, calcium and vitamin D intake are key nutritional factors that may act directly or indirectly on muscle and bone (Bass et al., 2005). However, in our study none of these variables were correlated with radial SOS and did not enter in the overall model as significant predictors of radial SOS. Muscle and bone are negatively impacted when there is nutritional deficiency, specifically when there is protein and energy deficiency (Bass et al., 2005). Our participants were healthy, typically-developing children without signs of protein and energy deficits. Therefore, it is not surprising that such variables did not enter in the regression models as significant predictors of radial SOS.

Calcium is a major constituent of bone and dietary calcium is thought to be an important determinant in maximizing bone mineral acquisition during growth (Bass et al., 2005; Cadogan et al., 1997; Valimaki et al., 1994). In the present study, we did find dietary calcium to be a weak but significant predictor of radial SOS but it only became a significant determinant of radial SOS after NTX was entered into the regression model. This suggests that the effects of calcium intake on the muscle-bone unit are modulated through bone resorption. Nutritional intake acts indirectly through endocrine factors on bone metabolism (modeling and remodeling) (Bass et al., 2005), which may explain the observed correlation between NTX and calcium intake, as well as the significant correlations of NTX with the relative caloric, protein and vitamin D intakes. Additional studies using bone formation and other markers of bone

metabolism, along with hormonal factors (particularly estrogen and IGF-1), are needed to clarify the relationship of dietary calcium and other nutritional components with the muscle-bone unit during growth.

4.4.4 Sex Differences

NTX was found to be the most significant predictor of radial SOS in boys but not girls, and could be explained by boys undergoing increased bone metabolism compared to girls. The development of cortical bone is sex-specific with the cortices of both sexes undergoing periosteal expansion and endocortical resorption before puberty, but with endocortical apposition during puberty in girls only (Daly et al., 2004; Garn, 1972; Kontulainen et al., 2006; Schöenau et al., 2000). Sex differences in proximal radial cortical density are thought to be the result of increased intracortical remodeling in boys (Schöenau et al., 2002), and could account for the strong presence of NTX as a predictor of radial SOS in the boys of our study.

In girls, we observed maturity to be the strongest predictor of radial SOS followed by calcium intake. It is likely that maturity was the strongest predictor of radial SOS in girls because on average they were found to be closer to their peak height velocity, thus more mature. The increased apposition of bone on the endocortical surface of bone in girls during puberty is believed to be a calcium reservoir for future reproduction and lactation (Garn, 1972; Kontulainen et al., 2006; Schöenau et al., 2001). Moreover, calcium intake was found to be significantly lower in girls and well below the estimated average requirement and recommended dietary allowance of 1100-1300mg/day (Ross et al., 2011). Thus, calcium intake was a significant predictor of radial SOS in our pubertal girls possibly because they were below the recommended daily levels.

Distal radial bone strength has been found to be more closely related to grip strength than to muscle CSA in both older men and women (Hasegawa et al., 2011), which is similar to our total group findings. However, when separated by sex, we found relative grip strength to be a significant predictor of radial SOS in boys only. Likewise, Lorebergs and colleagues (2011) found grip force to be a predictor of proximal radial strength in men, but not in women, for whom muscle size, i.e., muscle CSA, was a stronger predictor. Similarly, Vicente-Rodriguez and colleagues (2008), found grip strength to explain a larger percentage of variance in whole body BMC in boys compared to girls. Thus, it is possible that muscle function may be more important in males.

4.4.5 Limitations and Strengths

The major limitation to our study is that hormonal levels were not assessed and, with the collection of only urine samples, markers of bone formation were not measured. Thus, we cannot make conclusions regarding the contribution of osteoblast activity into the model. It could also be argued that QUS does not present discrete information on bone content, size, geometry or strength, but its SOS score reflects both the qualitative and quantitative properties of bone that contribute to its strength (Baroncelli, 2008). Importantly, transaxial QUS provides us with a measure reflecting bone strength that is independent of bone size, which is particularly important when investigating growing children.

Despite these limitations, ours is the first detailed study of the functional model of bone development in a cohort of healthy children to include both measures that reflect muscle and bone strength (rather than size), along with various modulators. An important strength of our study is that our measures of muscle and bone strength (relative grip strength and radial SOS)

are size-independent. Therefore, size is not a confounding factor in our muscle-bone interactions, which reiterates that muscle strength is a primary contributor to the development of radial bone strength. Another strength of our study was the examination of markers of bone metabolism, albeit only resorption. Finally, being able to identify these relationships using less advanced technology, not only demonstrates the strength of this proposed model of bone development, but also that QUS and simple measures of muscle strength are effective at examining the functional muscle-bone unit in children at the distal radius, which is a common fracture site in youth (Khosla et al., 2003). Evaluating bone health from the perspective of a functional muscle-bone unit may increase the sensitivity of fracture prediction.

4.5 Conclusions

The most important finding of this study is that, among 8-16 year old children and adolescents, muscle strength is related to bone properties (reflecting bone strength), above and beyond any other factor except for maturity, although the relative significance of these factors in predicting bone properties appears to be somewhat different between boys and girls. Dietary calcium was a weak but significant contributor of radial SOS but the effect of calcium on the muscle-bone unit is sex-specific (i.e., only in the girls) and may be modulated through bone resorption. Future studies examining the neuromotor properties of muscle in children are needed to help elucidate how muscle function influences the development bone strength, mass and geometry. Longitudinal studies measuring both muscle and bone strength are also needed to determine how the functional muscle-bone unit adapts with respect to changes in its modulators as a result of growth and maturation in boys and girls.

CHAPTER 5

Modeling the Changes in Bone Properties in Relation to Changes in Muscle Strength in Children across Puberty – a Longitudinal Study

5.1 Introduction

The functional model of bone development postulates that the primary mechanical challenges to bone's *mechanostat* during growth comes from increases in bone length and muscle force (Frost, 1987; Rauch & Schöenau, 2001). This suggests that the growth of bone and muscle are closely associated, and that bone must adapt its strength to withstand forces from muscle contractions (Schöenau & Fricke, 2008; Schöenau & Frost, 2002). Various cross-sectional studies have demonstrated positive relationships between bone properties and muscle mass, size or force in youth (Janz et al., 2015, Macdonald et al., 2006; Schöenau et al., 2000, 2002; Wang et al., 2007; Wey et al., 2011). Moreover, correlations between lean body mass and bone mineral content, which are surrogate measures of muscle and bone strength, have been found during growth and development (Faulkner et al., 1993; Manzoni et al., 1996), with a temporal association between muscle and bone development (Rauch et al., 2004). Research has demonstrated that the peak rate of increase in muscle mass (Rauch et al., 2004) and, therefore, muscle strength occurs after the peak rate increase in height (Blimkie, 1989), but before the peak accrual of bone mass (Rauch et al., 2004) and bone strength (Jackowski et al.,

2009), supporting the *Mechanostat Theory* notion of muscle mass or force driving bone strength. As the temporal accrual of muscle mass and bone mass is timed with the peak height velocity, it is likely that maturity plays a role in the development of the functional muscle-bone unit.

There are very limited data investigating longitudinal changes in the relationship between muscle and bone properties during growth in children. The majority of longitudinal studies have relied on growth velocities of muscle size or strength in relation to growth velocities in bone accretion or strength at the radius or tibia to support the hypothesis of developmental muscle bone interactions (Rauch et al., 2004; Ruff, 2003; Jackowski et al., 2009; Xu et al., 2009). Based on these growth velocities, the general consensus is that the changes in muscle size or strength precede changes in bone accretion or strength (Ruff, 2003; Rauch et al., 2004, Jackowski et al., 2009; Xu et al., 2009). The few longitudinal studies examining the muscle-bone unit have supported the aforementioned cross-sectional pubertal comparisons between muscle and bone strength at the upper and lower limbs (Wang et al., 2007; Wey et al., 2001).

Studies that have examined the muscle-bone unit did not use direct assessments of muscle function; instead, they use muscle size measures such as muscle cross-sectional area (MCSA) or lean body mass. This approach is based on the notion that muscle strength generally scales with muscle size and assumes appropriate measures of size are sufficient for muscle-bone unit investigations (Petit et al., 2005). Likewise, studies have used radiation-based technologies to indirectly measure bone strength through its properties (bone density, content, area). Transaxial quantitative ultrasound (QUS) measures the speed of sound (SOS) *along* the bone, making its assessment independent of bone size, allowing for better

comparisons between children of different sizes and ages (Baroncelli, 2008; Foldes et al., 1995). QUS measures reflect both quantitative and qualitative properties of bone (Jaworski et al., 1995; Prins et al., 1998), as the SOS measure is reflective of BMD, elasticity and microarchitecture of bone (Baroncelli, 2008). Specifically, the parameters are related to bone density and structure (Gluer et al., 1994) but not to cortical thickness (Njeh et al., 1999), which is an added advantage when working with youth or attempting to make meaningful comparisons with adults. Previous studies have demonstrated that QUS is associated with bone strength and can predict fracture risk, independent of bone mineral density in elderly subjects (Bouxsein et al., 1999).

This study examined the relationship between radial bone properties and forearm muscle strength in children and adolescents. More specifically, the study investigates whether changes in bone properties at a non-weight bone (i.e. radial SOS) are directly or indirectly related to changes in grip strength across boys and girls during the peri-pubertal period. A secondary purpose was to investigate the influence of maturation on the muscle-bone unit. The radius is measured in order to properly separate the influence of muscle properties on bone from other mechanical loads such as weight bearing or ground reaction forces. Grip force is a simple way to assess muscle strength at the forearm and has been found to be related to distal radial bone strength in older men and women (Hasewega et al., 2011). However, there has yet to be a study to examine the relationship between radial bone properties and forearm muscle strength in children and adolescents longitudinally. We hypothesized that changes in grip strength would significantly predict changes in bone properties in boys and girls during the peri-pubertal period, and that maturation would influence developmental changes in muscle and bone strength.

5.2 Methods

5.2.1 Study Design and Participants

Participants were part of the Brock Active Muscles Study (2010-2013) at Brock University in Ontario, Canada, and were recruited from local school boards in the Niagara Region, as well as through poster and information sessions at the University's recreation facility. The study utilized a mixed-longitudinal design, in which each participant was examined annually over a 2-year period. All procedures were reviewed and approved by the Brock University Research Ethics Board. Testing occurred during the spring and autumn months in order to avoid any seasonal effects typical of summer and winter months, particularly for physical activity (Riddoch et al., 2007). As there was an overlap in year, it is possible to estimate a consecutive 10-year developmental pattern (8-18 years) over a shorter study period (Baxter-Jones et al., 2003).

Eligible children had no history of chronic disease or medication use, and no medical conditions. Individuals with experiences affecting bone properties (i.e., use of steroid medication, growth delay, previous and/or current fracture) were excluded from the study. Adolescents with irregular menses or using oral contraceptives were also excluded. Written and informed consent was obtained from a total 172 parents and their children during the 4 years of the study.

5.2.2 Anthropometry and Maturity

Standing and sitting height were measured using a stadiometer (Ellard Instrumentation, Monroe, WA, USA) mounted to the wall and recorded to the nearest 0.1cm. Leg length was calculated by subtracting seated height (height minus sitting height) from standing height.

Body mass was measured to the nearest 0.1 kg using a calibrated balance beam scale (Zenith Digital Scale). Skinfold thickness was measured in triplicate at two sites (triceps and subscapular) using Harpenden calipers (British Indicators, Herts, England), and the median recorded. Adiposity (percent body fat) was estimated from the sum of these skinfolds, using age- and maturity-specific equations, as described by Slaughter et al. (1988).

The maturity offset (years from age of peak height velocity), an indicator of somatic maturity, was estimated using sex-specific regression equations (Mirwald et al., 2002). It was determined from measurements of height, seated height, leg length, body mass and chronological age (date of birth minus measurement date) and it was adjusted in accordance with the measurement at the age closest to the estimated age of PHV. All measurements were performed by the same investigator. The intra-operator coefficient of variation of the skinfold measurements in 10 children was 2.4% and the interclass correlation coefficient was 0.996.

5.2.3 Muscle Strength

Maximal dominant forearm strength was assessed by a hand-help dynamometer to determine maximal isometric grip force. The device handle was adjusted to the participant's grip and the test performed with the participant in a standing position with their dominant arm abducted at about 45 degrees elbow extended (Lorbergs et al., 2011). Participants were instructed to squeeze the instrument as hard as possible for 3 seconds. Measurements were recorded to the nearest 0.5 kg. Contractions were performed 3 times and the participant's largest value (best trial) was used to represent their absolute maximal isometric grip force. Proper technique was monitored in order to minimize postural compensations and corrected

as necessary. Isometric grip strength has been widely studied and reported for both genders throughout childhood and adolescence (Blimkie, 1989).

5.2.4 Bone Properties

Transaxial quantitative ultrasound (QUS, Sunlight Omnisense™ 7000S, Sunlight Medical, Israel) was used to assess bone strength by measuring the SOS (m/s) along the bone at the distal 1/3 of the radius. The strength of bone is determined by the shortest time elapsed between the transmission and reception of the signal transmission, with faster transmissions reflecting stronger bone (Njeh et al., 1999). The area of measurement for the radius was the midpoint between the olecranon process and the tip of the third phalanx; wide scans of 140 degrees around the radius were performed. All measurements consisted of at least three consistent cycles. A system quality verification of the QUS is performed with a Perspex phantom before the first test of each day. Although every effort was made for one operator to solely perform all QUS measurements for the duration of the longitudinal study, this was not always possible. Thus, the first operator performed almost all SOS measurements with an intra-operator coefficient of variation in 10 children of 2% and an interclass correlation coefficient of 0.98. The inter-operator coefficient of variation was 3%. *In vitro*, QUS has been shown to assess previously unquantified properties of bone fragility (Gluer et al. 1993), with measures reflecting both quantitative and qualitative properties of bone such as BMD, elasticity, micro-architecture, and structure (Baroncelli, 2008; Gluer et al., 1994; Jaworski et al., 1995; Prins et al., 1998).

5.3 Empirical Approach

5.3.1 Data and Preliminary Analysis

Repeated measures of participants bone properties, measured as radial SOS, grip strength and somatic maturity (i.e., maturity offset) resulted in a longitudinal data set consisting of 290 participant-measurement occasion observations. Table 5.1 presents the sex-invariant and sex-specific summary statistics across measurement occasions.

Table 5.1 Summary statistics per measurement occasion (number of observations, mean, SD).

	Obs (N)	Mean radial SOS	SD radial SOS	Mean Grip strength	SD Grip strength	Mean Maturity offset	SD Maturity offset
Measurement occasion							
1	129	3790	94	20.9	6.3	-1.32	1.86
2	115	3842	94	24.6	7.3	-0.40	1.97
3	46	3875	111	28.0	8.6	0.46	1.82
Total	290	3824	102	23.5	7.5	-0.68	2.00
	Girls						
1	65	3798	90	19.1	4.5	-0.66	1.56
2	58	3860	93	23.4	5.3	0.45	1.59
3	26	3902	101	25.5	4.8	1.12	1.32
Total	149	3841	101	21.9	5.5	0.08	1.67
	Boys						
1	64	3782	98	22.7	7.3	-1.99	1.92
2	57	3824	92	25.8	8.8	-1.27	1.95
3	20	3839	116	31.3	11.2	-0.41	2.03
Total	141	3807	100	25.2	8.9	-1.48	2.01

As expected, the mean radial SOS and grip strengths increased across time. Mean radial SOS increased 2.2% $\left(=\frac{3874-3790}{3790}\right)$ and the mean grip strength increased 34.0% $\left(=\frac{28-21}{21}\right)$, with similar increases for boys and girls. The distribution of boy and girl participants was similar (i.e., 149 boy and 141 girl participants). The likelihood ratio test does not reject the null

hypothesis that the sex-specific distributions across measurement observations are the same (p=0.749).

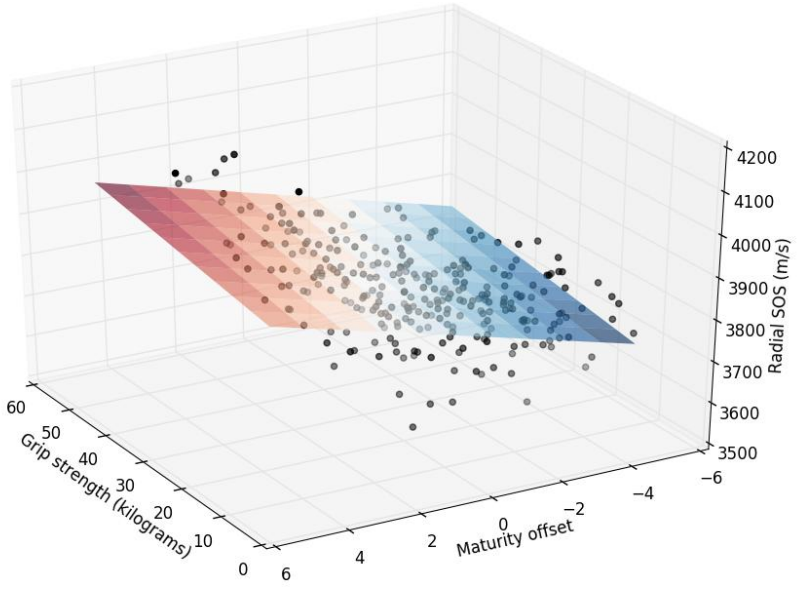

Figure 5.1 Three-dimensional scatter plot and projection plane
$(radial_{i,t} = 3795.11 + 1.86 grip_{i,t} + 21.66 maturity_{i,t}; r\text{-}squared = 0.28)$
Note: The plane is depicted in colours to aid in showing changes in the gradient. Lower values are presented in dark blue and higher values in dark red.

A cursory regression analyses based on the inclusion/exclusion of explanatory variables suggests that maturity-based model specifications explained more variation in the radial SOS than grip strength alone,, and an indirect maturity-effect potential exists via grip strength. Figure 5.1 presents the correlated relationships amongst the variables of interest. The base plot is a three-dimensional scatter plot with grip strength (x-axis), maturity offset (y-axis) and radial

SOS (z-axis). A projection plane based on the ordinary least squares overlays the scatter plot.[3] Both observation and the naive model results demonstrate strong positive conditional grip strength and maturity offset effects on bone properties.[4]

Figure 5.2 presents the two-dimensional scatter plots and best fit projection lines. Figure 5.2a plots maturity offset (x-axis) and radial SOS (y-axis) and Figure 5.2b plots the grip strength (x-axis) and radial SOS (y-axis). Grip strength and maturity offset effects are 209% $\left(2.09 = \frac{5.57 - 1.86}{1.86}\right)$, and 21% $\left(0.21 = \frac{26.16 - 21.66}{21.66}\right)$ larger than their associated effects under the coefficients of the plane equation. Figure 5.2c shows a positive maturity offset (x-axis) effect on grip strength (y-axis). Finally, the r-squared values indicate that maturity-based model specifications (Figure 5.1) explained more variation in the radial SOS (28%) than grip strength and maturity alone (17% and 27%, respectively; Figure 5.2). The sex-specific correlated summary analyses were similar.

[3] The subscripts i and t denote the i^{th} participant and the t^{th} measurement occasion. All coefficients are significant at the 0.05 level.

[4] Naïve model is used to refer to a model that does not account for the hierarchical data structure.

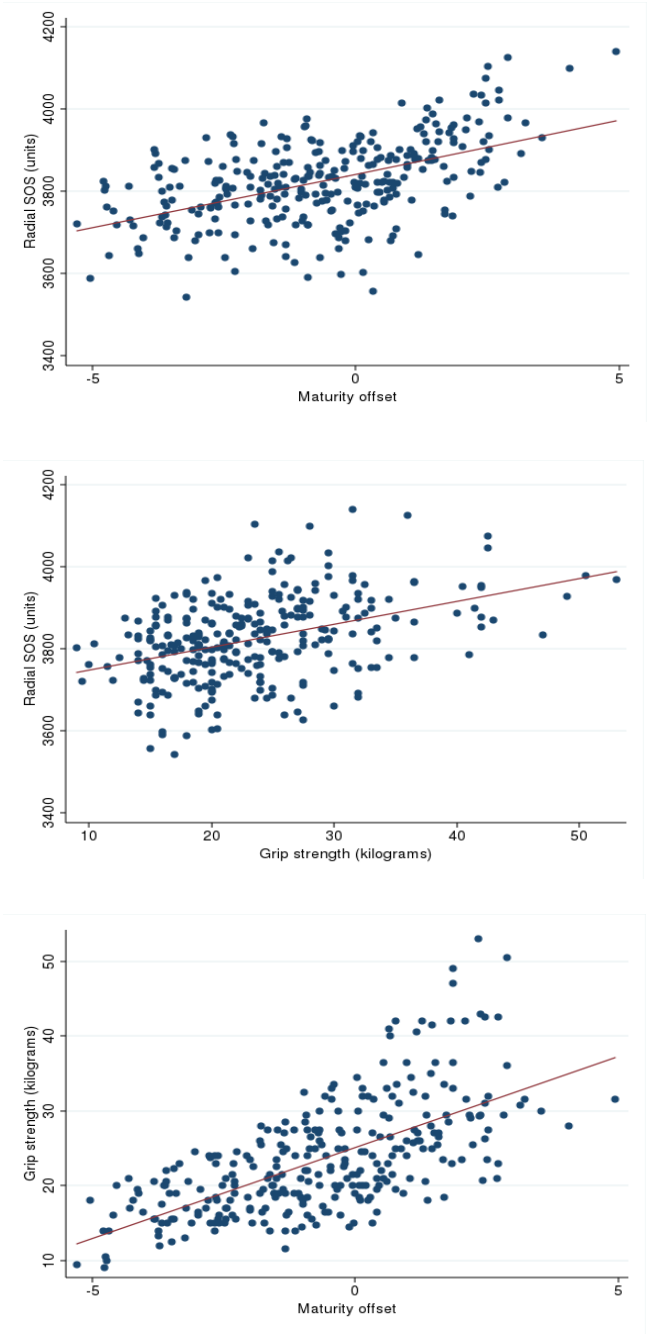

Figure 5.2(a-c) Scatter plots and lines of best fit for the total group.
$radial_{i,t}$ = 3841.86 + 26.16$maturity_{i,t}$; *r-squared* = 0.27
$radial_{i,t}$ = 3693.31 + 5.57$grip_{i,t}$; *r-squared* = 0.17
$grip_{i,t}$ = 25.13 + 2.43$maturity_{i,t}$; *r-squared* = 0.41
Note: 5.2a plots maturity offset (x-axis) and radial SOS (y-axis), 5.2b plots grip strength (x-axis) and radial SOS (y-axis), and 5.2c plots maturity offset (x-axis) and grip strength (y-axis).

5.3.2 Statistical Analysis

The empirical analysis implemented is a multilevel (random effects) hierarchical (participant and measurement occasion) structural equation (partial mediation) model that explicates the relationship between participants' grip strength and physical maturation on their bone properties. Using a random effects model allows the random intercept and slope effects to treat participants and grip strength as random samples from which to make population inferences. A participant's bone properties are expressed as:

$$bone_{i,t} = \left(b_0 + g_{0,i}\right) + b_1 grip_{i,t} + b_2\left(grip_{i,t}\,´\, g_{1,t}\right) + e_{i,t} \quad (1)$$

$$grip_{i,t} = \left(q_0 + g_{2,i}\right) + q_1 matu_{i,t} + m_{i,t} \quad (2)$$

where the subscripts i and t denote the participant and measurement occasion, respectively. The variable $bone_{i,t}$ denotes the i^{th} participant's bone properties in terms of radial SOS (m/s) at the t^{th} measurement occasion. The variable $grip_{i,t}$ denotes the participant's grip strength in kilograms, and $matu_{i,t}$ denotes his/her maturity offset value. The $\varepsilon_{i,t}$ and $\mu_{i,t}$ terms denote idiosyncratic residuals assumed to be uncorrelated and normally distributed with a mean of zero. The β and θ terms are the unknown fixed parameters, and the γ terms are the unknown random parameters. The random effects were assumed to be normally distributed with a mean of zero, and the covariates and residual terms independent of the random effects. No restrictions were imposed on the covariance structure of the random effects.

The model specification is very flexible in terms of accounting for the hierarchical data structure. Level-two random participant (i.e., intercept) components were included in both equations, and random grip strength (i.e., slope) component was included in the bone properties equation. The specification is a partial mediated model. Equation (1) represents a population

person's bone properties in terms of his/her grip strength, and equation (2) represents his/her grip strength in terms of maturity. The maturity offset variable was excluded from the bone properties equation. Its coefficient was not significant at the 0.05 level and the goodness-of-fit likelihood ratio test statistic did not reject the null hypothesis that the nested specification is a better fit (p=0.07).

The assumptions of normality were screened for by examining the kernel density plots of residual terms for the radial SOS and grip strength equations, and both appeared to be normal. On further examination, the Shapiro Wilks test did not reject normality for the grip strength equation but did for the radial SOS equation. However, we feel that our estimates are unbiased and efficient. Furthermore, any missing observations were dropped from the longitudinal empirical analysis.

5.4 Results

Table 5.2 presents the estimation results. The level 1 variances indicate that maturity increase grip strength, which in-turn, increase bone properties at the radius. The population maturity effect on grip strength was 3.46, and the grip strength effect on radial SOS was 10.60. A negative correlation between the intercept and slopes was found and may suggest that the effects of grip strength on radial SOS effects tend to be smaller in individuals with larger radial SOS measurements. An outcome of the mediate model specification is the ability to calculate indirect effects. Maturity had an indirect effect on bone SOS through grip strength (i.e., maturity affected grip strength, which in turn affected bone SOS). This indirect maturity effect $\left(\beta_1\theta_1\right)$ is 36.58.

Table 5.2 Multilevel mixed mediated regression results of grip strength, physical maturity offset, and sex on radial speed of sound (SOS).

	Total Group		**Males**		**Females**	
	Radial SOS					
Grip strength	10.6	[8.98,12.23]	6.60	[4.54,8.66]	14.26*	[11.96, 16.57]
Constant	3585.1	[3, 623.19]	3642.6	[3,700.37]	3536.4	[3, 585.83]
	Grip strength					
Maturity offset	3.46	[3.09, 3.82]	4.10	[3.62,4.58]	3.04	[2.68, 3.41]
Constant	25.88	[24.84, 26.92]	31.13	[29.83,32.42]	21.63	[20.84, 22.42]

*Significant sex differences ($p<0.05$); Unstandardized ß-coefficients are reported with 95% confidence intervals in parentheses. The coefficients are estimates of the impact unit changes.

A chow-test rejected the null hypothesis at the 0.01 level that the sex-specific data sets are the same suggesting that there is a difference in the mediation analysis between sexes. Boys' grip strength was greater (Table 5.1), and the marginal effect of maturity on it is greater than amongst girls, albeit not significant (4.10 versus 3.04). Girls appeared to have greater radial SOS levels than boys at measurement occasion 2 and 3 while boys appeared to have a transient dip in their radial SOS at measurement occasion 2 (Table 5.1), which corresponds to the time of peak height velocity (i.e. maturity offset=0). However, for females, the marginal effect of grip strength on radial SOS was significantly greater than that of males (14.26 versus 6.603). Boys and girls indirect maturity effects were 27.06 and 43.41, respectively.

5.5 Discussion

This is the first longitudinal study to implement an empirical analysis that allowed us to dissect both the direct and indirect effects of maturation on bone properties. During growth, there are age- and maturity-dependent changes to both muscle and bone properties. Previous longitudinal studies have demonstrated a close association between changes in muscle and bone mass or content during growth in children and adolescents (Faulkner et al., 1993; Janz et al., 2015, Macdonald et al., 2006; Manzoni et al., 1996; Rauch et al., 2004; Schöenau et al., 2000, 2002; Wang et al, 2007; Wey et al., 2011). However, it is not always clear how best to take into account the effects of maturity on the muscle-bone unit. When examining the functional model of bone development, it appears as though the primary challenge of growth on bone's mechanostat comes from changes in bone length as well as muscle forces (Frost, 1987; Rauch & Schöenau, 2001), which suggests that the effects of growth (i.e. maturation) should be mediated through these factors. Since muscle forces place the largest physiological loads on bone, and bone must adapt its strength to muscle contractions (Schöenau & Fricke, 2008; Schöenau & Frost, 2002), it is likely the largest influence of maturation would be mediated through changes in muscle forces. Traditional regression analysis, however, does not represent the relationships among the covariates and estimates their total effects.

Our model represented and attempted to better estimate the temporal relationships of a child's grip strength and physical maturation on his/her bone development. The fact that the maturity offset variable was excluded from the bone properties equation demonstrates modulating effects of maturation on bone that come indirectly from its association with grip strength, and directly from grip strength (Figure 5.3). The final r-squared values of the presented relationships between maturity, grip strength and radial SOS indicated that our

maturity-based model mediation specifications explain more variation in radial SOS (28%) than grip strength and maturity alone (17% and 27%, respectively). This is evident by comparing the r-sqaured values of the individual relationships with radial SOS (Figure 5.2a-c) with that of three-dimensional scatter plot (Figure 5.1). Specifically, the individual scatter plots (Figure 5.2a-c) showed a positive unconditional relationship between radial SOS and maturity, radial SOS and grip strength, as well as maturity and grip strength. The magnitude of the these relationships was different for grip strength and maturity, with their unconditional effects (coefficients) being greater than their conditional effects found in the plain equation (Figure 5.1). Furthermore, the percentage drop in the coefficient value from unconditional to conditional effects was much greater in grip strength (209%) when maturity is taken into account, compared to the drop in maturity effects (21%) when grip strength was considered. The larger decrease in grip strength coefficients when maturity is considered helps to show maturity's large influence on grip strength. This is also demonstrated by the larger r-squared value (0.41) in the relationship between grip strength and maturity (Figure 5.2c). The examination of the three-dimensional versus two-dimensional scatter plots shows that maturity was important to both bone and grip strength and supports the aforementioned maturity dependent role in the development of muscle-bone unit properties. Together, the large influence of maturity on grip strength and the larger r-squared value of the three-dimensional conditional model than the individual unconditional one with maturity only (0.28 vs. 0.27), alludes to a potential mediation effect between maturity and radial SOS.

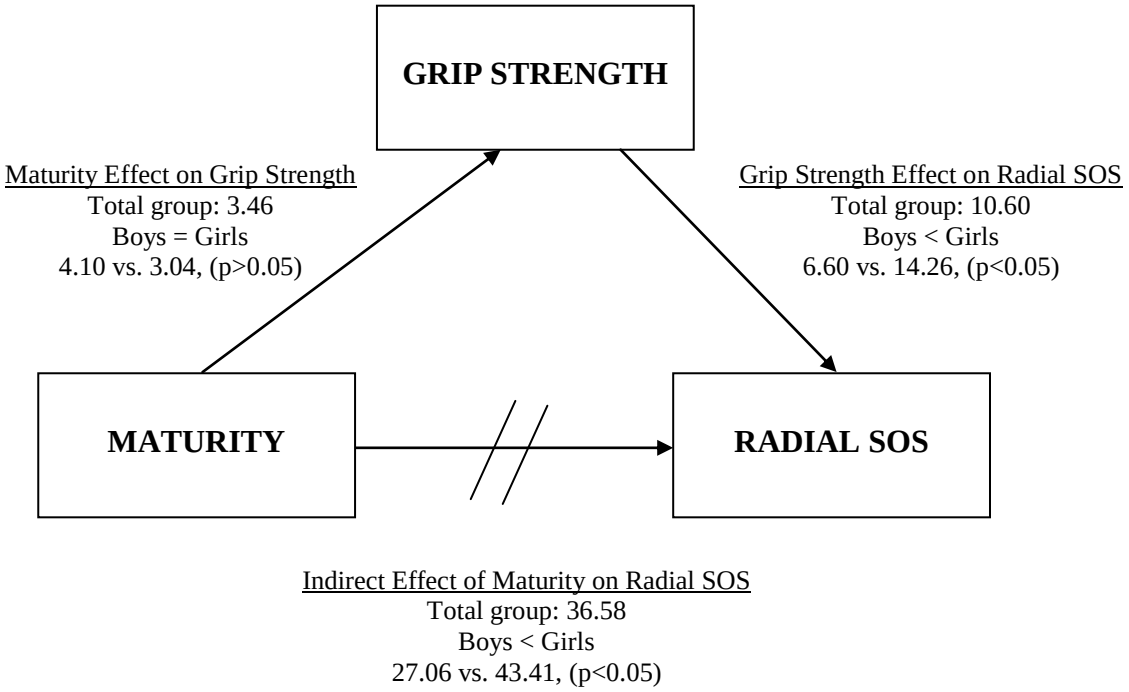

Figure 5.3 The mediating effects of maturity on radial SOS and its indirect association with radial SOS through grip strength in children across puberty.

Our partial mediation model represented and attempted to better estimate the temporal relationships of a child's grip strength and physical maturation on his/her bone development. This model attempted to tease out the complex relationship between a participants grip strength and their physical maturation on radial SOS, and the outcome of the mediated approach used was the ability to calculate the indirect effects of maturity on radial SOS through grip strength. The combination of the repeated measures and the highly correlated relationships between all three of these variables created an experimental environment that was well suited for a mediated approach because it attempted to untangle and explain the "potential" causal relationship between a person's grip strength and physical maturation on their bone strength. So this model provides us with direct and indirect maturity effects rather than the traditional total

effects and helps to clarify the nature of the relationship between the independent and dependent variables. By examining Figure 5.3 we can see there is no direct relationship between the independent variable (maturity offset) on the dependent variable (radial SOS), and the mediation model instead proposes that the independent variable (maturity offset) influences the mediator variable (grip strength), which in turn influences our dependent variable (radial SOS). The fact that the maturity offset variable was excluded from the bone properties equation demonstrated mediating effects of maturation on bone that come indirectly from its association with grip strength, and directly from grip strength (Figure 5.3). It has been suggested that when assessing bone properties with respect to muscle function, other variables such as maturity, may become less important (Macdonald et al., 2006; Petite et al., 2005). This is supported by our analysis finding that maturity's *significant* direct effect was reduced to an indirect effect on radial SOS that is being partially mediated through grip strength (Figure 5.3). One of the major factors that satisfies this mediation assumption is the known temporal association between maturity, grip strength and bone strength.

Longitudinally, it has been shown that accrual of muscle mass precedes that of bone mass (Jackowski et al., 2009; Rauch et al., 2004; Ruff, 2003), with peak lean tissue mass accrual preceding and predicting bone strength indices at the proximal femur during the pubertal growth spurt (Jackowski et al., 2009). This pattern of accrual was found to be similar between males and females, which suggests similar effects on how bone strength is amassed due to similar muscle mass accumulation in both sexes. Greater muscle area (Ruff et al., 2003) and lean mass (Wey et al., 2011) were observed to have greater developmental effects on bone strength, which suggests that stronger muscles contribute greater effects on bone strength. Muscle effects also tended to be greater in the upper as opposed to lower extremities, and

measured about 30% higher in the upper limbs after 2 years (Wang et al., 2007), with associations being greater later in adolescence in males than in females (Wey et al., 2011). However, these results may not be analogous to contributions of muscle strength gains on bone strength with maturation. Although the difference was not statistically significant, the influence of maturity on grip strength was found to be slightly greater in boys than amongst girls. This is not surprising as males typically have greater increases in muscle strength compared to females during adolescence, with marked sex differences later in adolescence particularly in the upper extremities (Blimkie, 1989; Parker et al., 1990; Round et al., 1999). These results are also reflected in our dataset when examining grip strength values in boys and girls by maturity offset (Table 5.1). The differing marginal effect of maturity on grip strength may have become significant between sexes had our age range extended beyond 15 years to include the period in which larger strength-related sex-differences occur (Jackowski et al., 2011). Males continue to grow in height until their early 20's, so that any later timing-effects of maturation may not have been observed in our current population of boys, and as a result our muscle-bone unit observations may be confounded by the truncated age range and in turn growth period in our males (Jackowski et al., 2011).

On the other hand, the advantage of the insignificant difference in the maturity effect on muscle strength between boys and girls is the ability to treat the indirect effect of maturation as constant (similar) in the two sexes. This underscores the greater direct effect of grip strength on radial SOS in girls, which implies that their bones are more responsive to muscle forces. This is similar to cross-sectional results by Janz et al. (2015) who also used a mediated approach and observed that muscle power accounted for a higher percentage of variance in tibial bone strength outcomes amongst adolescent females than in males. Moreover, our results are of

clinical importance because more women than men suffer from osteoporosis later in life (Hasserius et al., 2003). This geriatric disease has antecedents in youth, when improvements in bone properties are most advantageous to long term bone health (Heaney et al., 2000). The greater sensitivity of bone to muscle forces in females highlights potential areas for targeted interventions to improve muscle and, in turn, bone strength in this population. The radius is a common fracture site (Khosla et al., 2003), particularly in obese youth, who have been found to have a greater occurrence of radial fractures compared to their normal weight counterparts (Fornari et al., 2013). This makes muscle improving strategies important at a time when obesity rates are increasing (Ebbeling et al., 2002) and physical activity levels are declining (Trost et al., 2002). In general, our finding of significant effects of grip strength on radial SOS indicates potential protective mechanisms to improve bone properties in both sexes.

Typically males have been shown to have higher levels of DXA- and pQCT-derived bone properties and strength compared to girls (Janz et al., 2015; Macdonald et al., 2005& 2006; Schöenau et al., 2000, 2001, 2002a, 2002b). Conversely, radial SOS values have been shown to be higher in females rather than males (Christoforidiset al., 2009; Zadik et al., 2003), with SOS values increasing with age in both sexes (Barconelli, 2008; Klentrou & Ludwa, 2011; Zadik et al., 2003). Similar to previous literature, we also found radial SOS to be greater in girls than boys, with differences increasing with maturity, and this is likely due to the transient dip in radial SOS that we observed in the boys around the time of puberty. This temporary decrease in male radial SOS around the time of peak height velocity is another possibility for the discrepancy in the observed sex differences in the marginal effects of grip strength on radial SOS. Cross-sectionally, a transient decrease in BMD has been reported in both males and females during peak linear growth, which is suggested to result in a greater incidence of

fractures at this time (Faulkner et al., 2006). It is possible we do not see this drop in SOS values in girls due to the added sensitivity of their bones to grip force (Schöenau et al., 2000, 2002). It is also possible that there are other protective influences such as estrogen, which has been shown to lower the mechanostatic threshold in females, which then makes bones more responsive to stimuli (Frost, 1999, 2000).

This lowering of the mechanostatic threshold may help to explain why we find a negative correlation between the intercept and slopes, which suggests that individuals with larger radial SOS measurements tend to have smaller grip strength effects. Daly et al. (2004) demonstrated that, independent of age, there was more bone (mass and cortical area) for a given muscle area in post-puberty compared to pre- and peri-puberty. In addition, during puberty, the bone/muscle ratio increases in girls but not in boys (Daly et al., 2004; Schöenau et al., 2000). Thus, it is possible that there is something else contributing to radial bone properties in those with higher SOS values. Other hormonal or nutritional factors could be modulating this muscle-bone relationship and longer longitudinal analyses are needed to see if this negative slope intercept interaction is sex and/or maturation specific and reflects the aforementioned cross-sectional results of muscle-bone interactions.

A limitation of this study is that other moderating factors of bone strength such as physical activity, and nutrition were not included in the model due to multiple missing values over the 3-year data collection. Another limitation is that we followed each participant for only 2 years, with smaller sample sizes of individuals for whom we have 3 observable time points. Furthermore, extending our range beyond 15 years of age would have helped to provide a clearer indication of maturational effects and potential sex differences due to the later maturation in males.

The strength of our study comes from the model we have implemented to statistically represent the functional model of bone development and the proposed physiological effects of muscle force on bone during growth and maturation. Furthermore, ours is the first study to *longitudinally* assess measurements of both muscle function and bone strength, as opposed to surrogate strength measures of muscle (muscle size or lean body mass) and bone (bone mineral density, content or area). To our knowledge, this is the first study to use mediation analysis in a longitudinal approach to characterize a temporal sequence of maturation, muscle force and bone strength. This type of analysis is advantageous as it allows us to elucidate the common effect of maturation on muscle and bone separately, as well as its combined effect on the muscle-bone unit as a system. The limitation of cross-sectional analyses is that individuals may have unobservable characteristics that can potentially impact results. The advantage of using a hierarchical model in this longitudinal design is that there are many participants with multiple time points, and by imposing the partially mediated structural equation model we are able to account for these unobservable characteristics. Furthermore, what is helpful is that the model is set up in such a way that there are no restrictions imposed, thus permitting for participants and grip strength to remain random and allowing us to take into account the potential variability between and within participants.

To date only one other study (Janz et al., 2015) has used mediation analysis, albeit cross-sectionally, to observe pQCT-derived tibial bone strength from MCSA mediated lower body muscle power in adolescent males and females. The functional model of bone development (Rauch & Schöenau, 2001) is a complex model with multiple direct and indirect pathways in which variables can influence bone strength. Therefore, there is a need for more

mediated types of analysis, particularly from a longitudinal perspective, to further elucidate the ways in which determinants of bone strength impart their influence during growth.

5.6 Conclusions

This study implemented a multilevel hierarchical structural equation model to examine if changes in radial SOS are directly related to changes in forearm muscle strength in boys and girls during the peri-pubertal period. Somatic maturity was found to be empirically important to both bone properties and grip strength. This is why the maturity-based models explain more variation (28%) in the radial SOS than grip strength and maturity alone (17% versus 27%), suggesting that the effect of muscle force on bone properties is modulated by physical maturation. One interesting finding is that the influence of grip strength was small in individuals with high radial SOS values. Another interesting finding is that males appear to have a transient dip in their radial SOS around the time of peak height velocity. Finally, the effect of grip strength on bone properties is stronger in girls, which means that their bones may be more responsive to muscle forces.

CHAPTER 6

General Discussion

6.1 Summary of Major Finding

This research was carried out in two parts. The first part was a systematic review of the literature on the effect of whole body physical activity on the bone development in children. The aim was to find all available randomized control trials and controlled studies in order to examine the benefits of any type of physical activity interventions on bone status in healthy (non-clinical, non-athlete) children and adolescents 6 to 17 years of age. For this purpose, we included all types of bone parameters from various bone assessment techniques as primary outcome measures, provided that there were at least two measurement time points. A computerised search of the MEDLINE and PubMed databases was performed using a comprehensive combination of keywords to describe exercise, bone and participant parameters. This review adds to previous literature by discussing and including less traditional, both static and dynamic measures of bone, such QUS and biochemical markers of bone metabolism, respectively. In addition, it provides additional information as to the best *time* during maturation to expect changes in bone properties, and which *type* of activity is best at eliciting bone adaptations within each pubertal stage. The analysis showed that for long-term gains, short-duration, high-impact exercises undertaken early in childhood (pre and early puberty) and sustained into adulthood has a persistent effect on bone over and beyond that of normal growth and development.

Furthermore, our systematic review found that the mechanical loads experienced by bone come through either physical activity or muscle contraction, and that studies need to begin relating bone strength to muscle function, a functional muscle bone unit. This sentiment is shared by the narrative of Tan et al. (2014) expressing the need to separate the loading influences of physical activity and muscle function. This narrative motivated us to conduct two observational studies that investigated the influence of muscle properties on bone strength apart from weight-bearing or ground reaction forces. Tan et al. (2014) also emphasized the importance of the independent role of muscle on bone and its association with sex, maturation and physical activity be considered in studies with children and adolescents in order to provide a comprehensive picture of mechanisms that drive bone adaptations. These are the factors, in addition to nutrition and bone metabolism, that we attempted to investigate in Part 2 of this dissertation. Taken together, this updated systematic review and recommendations of Tan et al. (2014), underscore the theoretical framework we used to conduct the cross-sectional and longitudinal studies in the second part of this dissertation.

The overall finding of the first, cross-sectional study was that relative grip strength, together with maturity offset, dietary calcium and NTX, explained up to 21% of the variance in radial SOS in this cohort of children. Specifically, muscle force was related to radial SOS, above and beyond any other factor except for somatic maturity. It was expected that bone resorption, physical activity and dietary calcium would be correlated with radial SOS. However, physical activity was not found to be a significant predicator of radial SOS. NTX was also found to be an explanatory variable in the observed variance of radial SOS, with calcium only entering into the regression model after accounting for NTX. This finding would suggest that the influence of dietary calcium may be mediated by bone metabolism and may

impart its effects on bone properties through the regulatory feedback loop proposed in the functional model of bone development. It was hypothesized that muscle force would be positively associated with bone properties in boys and girls. Size-adjusted grip strength was found to be a significant predictor of radial SOS in boys only, however, which suggests that muscle function may be more important in males. In addition to muscle force, NTX was a key predictor of radial SOS in boys but not girls. On the other hand, dietary calcium was a predictive variable in girls but not in boys. Regardless whether the analysis was conducted separately by sex or combined, maturity offset was always found to be a strong significant predictor of radial bone strength. The results of this cross-sectional study demonstrate that the relative significance of factors predicting radial SOS appear to be somewhat different between sexes during peri-pubertal years.

In order to elucidate whether radial bone properties would change as a function of muscular changes during growth, we examined the muscle-bone unit over a 3-year period in boys and girls between the age of 8 to 15 years. The longitudinal findings in the second study supported the hypothesis that grip strength would increase with growth and that these changes would predict positive increases in radial SOS. Maturity was found to have positive direct effects on grip strength, which in-turn, increased the SOS at the radius. The maturity-based mediated model explained more variation in radial SOS than grip strength alone, which suggests that maturity is important to the development of both bone and muscle strength. Furthermore, we have shown that grip strength contributes to radial SOS above and beyond maturity, which further supports the concept of the muscle-bone unit. Contrary to the cross-sectional analysis, the effects of grip strength on radial SOS was found to be significantly greater in girls, however, which suggests that their bones are more responsive to muscle forces

than those of boys. There were two notable results observed in the longitudinal study. Firstly, the influence of grip strength was small in children with high radial SOS values. Secondly, boys only showed a transient dip in radial SOS around the time of their peak height velocity. These findings demonstrate that the muscle-bone unit relationship changes with maturity, and may reflect some of the muscle-bone unit sex differences observed in the cross-sectional analysis that are maturity-related.

Overall, the second part of this dissertation provides support for the functional model of bone development from both a cross-sectional and longitudinal perspective. Together, they provide a "big picture" view of the development of the muscle-bone unit during maturation in boys and girls. The first study provided support by assessing the muscle-bone unit using simple measures of muscle force and bone properties in both boys and girls. Moreover, for the first time, there was an attempt to include an indicator of bone turnover, specifically resorption, which is a central aspect of bone regulation in the feedback loop proposed in this model. Most often this model is examined from a static rather than dynamic perspective. The second study provided additional support by applying a mediated statistical model to help examine the complex direct and indirect pathways demonstrated in the functional model of bone development. It again measured *both* muscle force and bone properties in both boys and girls over time, which was something that had yet to be investigated in the peri-pubertal population. Furthermore, it helped to explain the role of maturation and its influence on the functional model of bone development.

6.2 Theoretical Development/Framework

The overall goal of this dissertation was to increase the understanding of the normal development and adaptation of human bones, particularly regarding the adaptation to muscle function in boys and girls during puberty. Bone development is a product of a complex interaction between genetic and environmental factors, which includes diet and mechanical loading through physical activity (Gordon, 2003; Steelman & Seitler, 2001). Different types of physical activity such as resistance training (Nichols et al., 2001) and weight-bearing exercise (Fuchs & Snow, 2002; Lehtonen-Veromaa et al., 2000) have been shown to have positive loading effects on developing bone mass and strength through muscle contraction and ground reaction forces, respectively. The timing of application of these particular loads is also very important. Physical activity at a young age has been shown to have positive influences on bone properties later on in life (Davies et al., 2005), with bones being more responsive to certain exercise prescriptions early on in puberty. This finding suggests a *window of opportunity* for bone response (MacKelvie et al., 2002).

Evidence that supports the role of physical activity on bone development has been accumulated from a wide range of studies that investigated different activities, using various intervention methods and bone assessment techniques, across a wide range of ages in children and adolescents. This led to the primary objective for the first part of this dissertation, which was to conduct a systematic review of the literature concerning the effect of physical activity interventions on the development of different bone properties in healthy (non-clinical, non-athletic) children and adolescents, 6-17 years of age. The purpose of this review was to help ascertain the best time to introduce physical activity, and determine if there was a specific modality that was best suited to improving bone strength during growth and development. The

analysis showed that regular exercise can be an effective way to improve bone density, size, shape, and strength, and that regardless of sex, skeletal location and type of activity, the greatest gains in bone properties were during the early pubertal years. This finding suggests that the pubertal period may be the best time to generate skeletal adaptations to physical activity. Regardless of pubertal stage, the *duration* of the intervention and the *intensity* of the activity was important to the *type* of activities utilized. Shorter duration interventions (8-10 months) utilizing jumping activities with high ground reaction forces were effective in demonstrating positive bone changes (Bass et al. 2007; Fuchs et al., 2001; MacKelvie et al., 2001, 2002; McKay et al., 2005; Petit et al., 2002; Weeks et al., 2008), with longer duration interventions (10-24 months depending on pubertal stage) being needed for weight-bearing and resistance training activities (Alwis et al., 2008a; Courteix et al., 2005; Linden et al., 2006, 2007; Morris et al., 1997; Nichols et al., 2001; Stear et al., 2003; Valdimarsson et al., 2006). Most importantly, it appeared that short-duration, high-impact exercise undertaken in early childhood had lasting positive effects on bone, over and beyond that of normal growth and development.

It was clear from the systematic review that physical activity has positive effects on bone development in children and adolescents. Furthermore, habitual physical activity has been shown to enhance not only bone accrual (Baxter-Jones et al., 2003) in youth, but lean mass (Baxter-Jones et al., 2008) as well. Both bone accrual and lean mass are important to promote musculoskeletal health. It is not sufficient, however, to examine bone development alone; one must also consider muscle development and its interaction with bone and treat the development of these two physiological tissues as a system, or a muscle-bone unit. In fact, some of the largest loads placed on bone come from muscle contractions (Rauch et al., 2004; Schöenau &

Frost, 2002). It can be difficult, however, to discern whether muscle contractions or ground reaction forces provide the greatest adaptation to bone strength as they are not always mutually exclusive.

One way to examine the effects of these forces separately is to investigate bone properties of the non-weight bearing versus weight bearing bones. This reasoning led to the second part of this dissertation, in which both a cross-sectional and longitudinal design were used to examine how muscle growth and function influence bone properties in children, apart from the effects of weight-bearing or ground reaction forces. This was achieved by investigating the radius, which is not a weight-bearing bone. Using this bone allowed us to fully partition the effects of muscle function from other loading forces by measuring muscle size and force as well as assessing physical activity, diet, and bone resorption. As introduced in Chapter 1, the research approach chosen was based on the functional model of bone development proposed by Rauch and Schöenau (2001), which, in turn, is based on the Mechanostat Theory. According to the theory, there is a physiological set point at which bones adapt their strength to the external forces applied to them (Bailey et al., 1996; Frost, 1987). The model of bone development (see Figure 2.1, Chapter 2) postulates that the primary mechanical challenges to bone's *mechanostat* during growth comes from increases in bone length and muscle force (Frost, 1987; Rauch & Schöenau, 2001). Other factors known to affect bone, such as hormones, physical activity, and nutrition, can help or hinder the muscle-bone unit relationship, with bone metabolism regulating the balance between bone strength and deformation (Rauch & Schöenau, 2001; Schöenau, 2005a, 2005b).

The primary purpose of Part 2 was to use an observational approach to assess functional changes in the muscle-bone unit in order to determine if changes in radial bone properties are

influenced by muscle strength changes that result from normal growth and maturation in peri-pubertal children. To this end, we followed children throughout their critical peri-pubertal years, over a 2-year period. The first study sought to evaluate the functional model of bone development (Rauch and Schöenau, 2001) cross-sectionally, by examining the relationship between muscle characteristics (size and strength) and radial bone properties, along with the influence of physical activity, nutrition, and bone resorption on this relationship in children and adolescents. For this study, we adapted the functional model of bone development proposed by Rauch and Schöenau (2001) to correspond with the variables we were attempting to investigate (Figure 6.1). The cross-sectional examination was followed by a longitudinal investigation of the muscle-bone unit in the same cohort of children followed for 2 years (3 measures). This study sought to build on the cross-sectional results to determine whether the association between muscle force and radial bone properties was co-modulated by physical maturation.

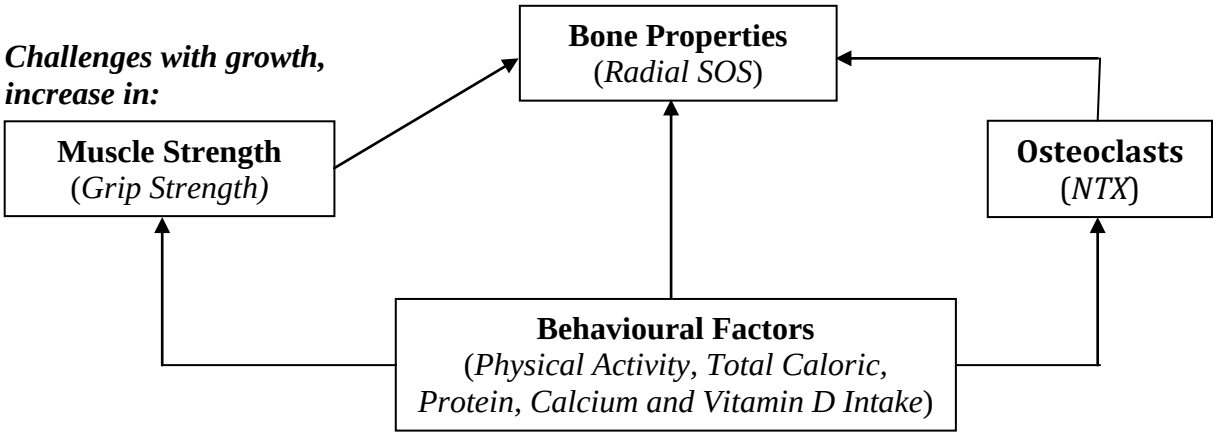

Figure 6.1 Adapted model of functional bone development (based on the model by Rauch & Schöenau, 2001).

6.3 Cross-sectional versus Longitudinal Functional Model of Bone Development

6.3.1 Maturation

Maturation is a variable process (timing and tempo) experienced by all individuals, which does not always proceed at the same rate as chronological age (Malina et al., 2004). It was, therefore, not a surprise that maturity offset, which we used as an alternative to chronological age, was found to be a significant predictor of radial SOS in both cross-sectional and longitudinal studies. LBM and BMC are closely associated during growth and maturation (Manzoni et al., 1996; Wolfe et al., 2006), with the most rapid accretion of BMC occurring around the pubertal growth spurt (Bonjour et al., 1991, Thientz et al., 1992). Moreover, the peak rate of muscle mass accrual (Rauch et al., 2004), and therefore strength, occurs after the peak increase in linear height (Blimkie, 1989), but before peak accrual of bone mass (Rauch et al., 2004) and strength (Jackowski et al., 2009). Therefore, we see a temporal association between maturity, muscle and bone development.

Maturity offset was the only variable consistently found to be a significant predictor of radial SOS in all cross-sectional regression models regardless whether it was conducted for the total cohort or separated by sex. In the longitudinal analysis, the maturity-based mediated model explained more variation in radial SOS than grip strength alone, which suggests that maturity plays a role in the development of both bone and muscle strength. It has been suggested that, when assessing bone strength with respect to muscle function, other variables, such as maturity, may become less important (Macdonald et al., 2006; Petit et al., 2005). This suggestion is supported by our empirical analysis that excluded maturity offset from the bone properties equation due to its loss of significance, while a partially mediated model showed

maturity having an indirect effect on radial SOS through grip strength. Comparing the effects of maturity on radial SOS between the cross-sectional and longitudinal studies may help to explain some of the differing sex-related muscle-bone unit relationships observed between studies, and between cross-sectional group analyses (total versus sex-specific). This complex inter-relationship is shown in Figure 6.2 through graphing longitudinal radial SOS, grip strength and maturity offset trends against the maturity offset applied during cross-sectional analyses. Examined this way, the observed differences in the muscle-bone unit across studies and sexes appear to be related to maturity, since maturity was found to be significantly different between boys and girls.

An important difference between the two studies is that grip strength was found to be a significant predictor of radial SOS in boys but not girls cross-sectionally, yet when analyzed longitudinally, the direct effect of grip strength on radial SOS was greater in girls compared to boys. Previously, distal radial bone strength was found to be closely related to grip strength in both older men and women (Hasegawa et al., 2011). Likewise, cross-sectionally, grip force was found to be a predictor of proximal radial bone strength in men but not in women. For women, muscle size was a stronger predictor of proximal radial bone strength (Lorebergs et al., 2011). The influence of maturity on grip strength was found to be greater in males than in females, although the difference was not statistically significant. The standardized grip strength trend lines in Figure 6.2 show greater grip strength values in males compared to females, especially later on in maturity. This finding is supported in the literature with males typically having greater increases in both muscle size and strength compared to females during adolescence, particularly later in puberty and in the upper extremities (Blimkie, 1989; Parker et al., 1990; Round et al., 1999).

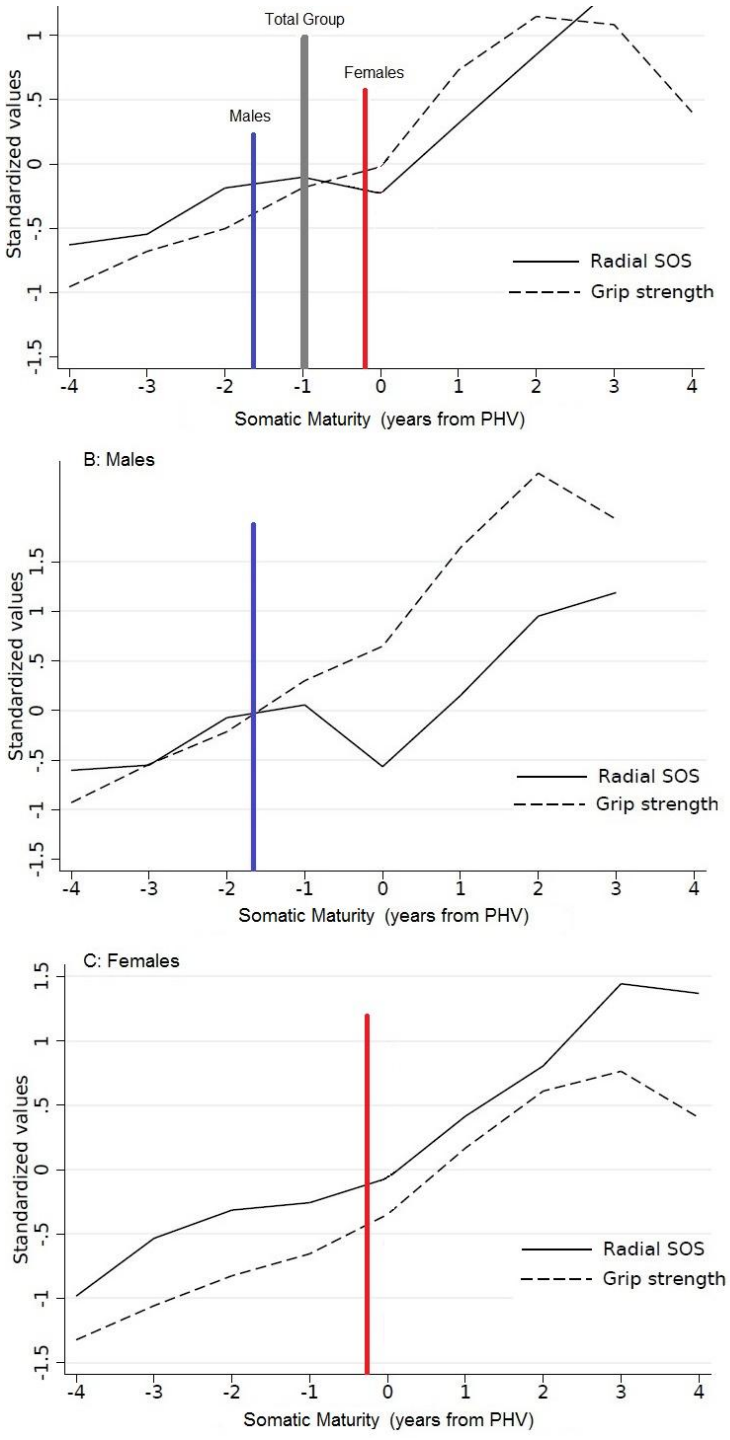

Figure 6.2 Radial SOS and grip strength and somatic maturity trends. Vertical lines correspond to cross-sectional average years from peak height velocity values.

It is possible the discrepancy in the marginal effects of grip strength on radial SOS between sexes is due to the transient dip in male radial SOS observed around the time of peak height velocity (Figure 6.2b), which demonstrates a disassociation between the increasing radial SOS and grip strength trend lines across maturity. Figure 6.2c shows a similar pattern in the increasing trend line of grip strength and radial SOS in girls, but not boys (Figure 6.2b). When overlaying the cross-sectional maturity offsets onto the longitudinal trends, the male maturity offset (-1.7 years) corresponds to an increasing radial SOS and grip strength trend line for both the total group and males (Figure 6.2a and 6.2b, respectively), which makes it appear as though they are associated. Conversely, the female maturity offset of -0.2 years corresponds to a dip in radial SOS trends for the total group (Figure 6.2a) and similar patterned increase in radial SOS and grip strength trend lines for females (Figure 6.2c). It is unlikely that the grip strength's diminished role in the cross-sectional regressions for females is related to this small dip in the observed total group radial SOS trend line (Figure 6.2a); this drop actually corresponds to the larger observed decrease in radial SOS scores in males at this time. Moreover, since the cross-sectional maturity offset of -0.2 years is almost at the point of PHV in girls, may explain why maturity is the strongest predictor of radial SOS cross-sectionally, and why grip strength does not enter into the regression model. These results demonstrate that a cross-sectional study does not paint a clear overall picture of the development of the muscle-bone unit across maturation, and may not reflect what is actually occurring longitudinally over time.

It is important to note that some of the aforementioned differences in the relationship between muscle strength and bone properties between studies could be due to how muscle force was presented. Muscle force was presented in relative values, as grip strength corrected

for forearm cross sectional area in the cross-sectional study. In the longitudinal study, absolute grip strength was used. In the cross-sectional study, absolute grip strength was expected to be a significant determinant of radial SOS, and as such, was originally entered into the model. We found absolute grip strength to not have any influence on radial SOS, and as a result, the next step was to investigate size-corrected grip strength. Unlike absolute grip strength, it was observed to be a significant predictor in our regression models. It is possible that relative grip strength entered into the models as opposed to the absolute grip strength as it was a better measure of muscle function than absolute grip strength alone. Moreover, it is not completely surprising that when grip strength was corrected for muscle size, in the cross-sectional analysis, that grip strength was found to be significant predictor of radial SOS in boys only. The smaller increases in grip strength due to maturity in girls would have been normalized when taking muscle size into consideration. Research has demonstrated a plateauing effect of muscular strength in females with growth when normalizing for body mass or muscle size (Blimkie, 1989; Malina et al., 2004).

We followed the same procedure for the longitudinal study by investigating the effect of absolute grip strength on radial SOS first. Again, this was due to our expectation that grip strength would be a significant predictor of bone strength. One of the purposes of the longitudinal study was to investigate how maturation influenced the functional model of bone development by investigating its influence on muscle and bone development separately and together. As the changes in muscle size are greatly influenced by maturation, correcting for muscle size, like in the cross-sectional study, may have only taken into account the effects of maturity on muscle strength without considering its influence on radial SOS.

Our cross-sectional results imply that muscle function may be more important to radial bone strength in males, while the longitudinal results suggest that female radial bones are more responsive to muscle forces. With respect to maturity, we found maturity to be a stronger predictor of radial in girls compared to boys cross-sectionally. This result is also reflected in the longitudinal analysis with the indirect effect of maturity on radial SOS being greater in girls compared to boys (Figure 5.3). Perhaps the maturity-dependent muscle size effects are inadvertently taken into account during the mediated effects of grip strength on radial SOS in the longitudinal analysis. Beyond muscle size, neuromuscular properties may help to account for increases in muscle strength and function with maturation (Blimkie, 1989), and may explain the sex-related difference in the relative vs. absolute grip strength predictor of radial SOS between studies.

6.3.2 Bone Resorption and Calcium Intake

The central aspect of bone regulation in the functional model of bone development (see Figure 1.1 in Chapter 1) is the regulatory feedback loop between bone deformation (tissue strain) and bone strength, which is controlled by osteoblasts and osteoclasts (Rauch and Schöenau, 2001). The majority of studies examining the muscle-bone unit do so from a static perspective and fail to take into account the dynamic contributions of bone cells to the model. Instead, imaging technologies are used to infer cellular actions by measuring changes to periosteal and/or endocortical bone surfaces (Daly et al., 2004; Macdonald et al., 2006; Schöenau et al., 2000). The cross-sectional study in this dissertation is the first to measure a marker of bone resorption within the context of the functional model of bone development in

healthy children. As hypothesized, NTX was found to be a strong significant predictor of radial SOS after taking into account maturity, grip force, physical activity and nutritional influences.

When analyses were run separately by sex in the cross-sectional study, NTX was found to be a significant predictor of radial SOS in boys only. This cross-sectional finding can be put into perspective when radial SOS trends are examined longitudinally. It is possible NTX entering the male regression model in the cross-sectional analysis reflects the observed drop in radial SOS males that was observed in longitudinal study (Figure 6.1b), and may explain why the increasing trend for radial SOS remains below that of grip strength in males after the age of peak height velocity (Figure 6.2c). This result also reflects the temporal development of the muscle-bone unit and provides evidence that increases in muscle strength occur prior to increases in bone.

The development of cortical bone is sex-specific. Sex differences in cortical density at the proximal radius may be due to increased intracortical remodeling in boys (Schöenau et al., 2002), which accounts for the strong presence of NTX in the male regression model. Cortical bone undergoes periosteal expansion and endocortical resorption before puberty, and endocortical apposition during puberty in girls only (Daly et al., 2004; Garn, 1972; Kontulainen et al., 2006; Schöenau et al., 2000). At least half of all adult mineralized calcium is laid down during the adolescent years (Bonjour et al., 1991, Thientz et al., 1992), and the increased apposition of bone on the endocortical surface of bone in girls during puberty is believed to be a calcium reservoir for future reproduction and lactation (Garn, 1972; Kontulainen et al., 2006; Schöenau et al., 2001). This supports our finding that calcium intake was a significant predictor of radial SOS in girls only, in addition to maturity offset, in a cohort of females who, on average, were at the age of peak height velocity. Moreover, during puberty, the bone/muscle

ratio increases in girls but not in boys (Daly et al., 2004, Schöenau et al., 2000) due to this increase in bone accrual (mass and cortical area) for a given muscle area post-puberty (Daly et al., 2004). This increase in bone accrual may correspond to the consistently higher longitudinal trend of radial SOS relative to grip strength in females (Figure 6.1c).

The increased endocortical apposition in adolescent females is believed to be the result of the lowering effect of estrogen on the mechanostatic threshold, which makes female bones more responsive to stimuli (Frost, 1999, 2000). The lower mechanostatic threshold may explain why longitudinally grip strength had larger effects on radial SOS in girls compared to boys, and why larger radial SOS measurements tended to have smaller grip strength effects. Lastly, the presence of NTX and calcium intake in the cross-sectional male and female regression models, respectively may help to explain the presence or absence of grip strength in those models. Grip strength may only be a significant predictor of radial SOS in males to compensate for NTX entering into their model due to the dip in radial SOS. On the other hand, the absence of a strong grip strength effect in females may be due the much stronger association of maturity and calcium intake in that population. It would have been interesting to see if a marker of bone formation, had it been measured, would enter the female regression model with calcium intake to support this increased bone apposition and longitudinal trend of higher radial SOS relative to grip strength, in the same way NTX entered the male regression model to reflect the observed dip in radial SOS.

Calcium is a major constituent of bone and dietary calcium intake is thought to be an important determinant in maximizing bone mineral acquisition during growth (Bass et al., 2005; Cadogan et al., 1997; Valimaki et al., 1994). Calcium intake was hypothesized to be a significant independent predictor of radial SOS. However, it only became a significant

predictor after NTX was accounted for in the total group cross-sectional regression model. Thus, the influence of dietary calcium may, in fact, be mediated by bone metabolism. Nutritional intake acts indirectly through endocrine factors on bone metabolism (modeling and remodeling) (Bass et al., 2005), with calcium intake potentially imparting its effects on radial SOS through the regulatory feedback loop proposed in the functional model of bone development. Unfortunately, neither NTX nor calcium intake were included in the longitudinal analysis, and therefore we cannot deduce their direct or indirect mediated impact on the functional development of the muscle-bone unit over time.

6.3.3 Physical Activity

It was not a surprise that physical activity was not a significant predictor in the variance of radial SOS, because of the design of the two observational studies using a non weight-bearing bone. Habitual physical activity has been shown to enhance bone accrual (Baxter-Jones et al., 2003) in youth, however physical activity was found to be negatively associated with radial SOS. This negative association is likely due to the concomitant increase in SOS and the decrease in physical activity levels with increasing maturation. This physical activity trend has consistently been described in the literature: Low levels of physical activity are evident among contemporary youth (Strong et al., 2005), and activity levels decline from childhood to adolescence, particularly in girls (Sallis et al., 2000, Sherar et al., 2007, Trost et al., 2002). There is a close correlation between LBM and BMC during growth and maturation (Manzoni et al., 1996; Wolfe et al., 2006), which makes it possible for physical activity to influence bone properties through its ability to enhance lean mass during this time (Baxter-Jones et al., 2008). Since physical activity was not significant in the cross-sectional analysis, and for this reason

was not included as a variable in the longitudinal analysis, it is not possible to speak to the potential direct effects of activity on radial SOS, or the indirect effects on radial SOS mediated through grip strength improvements over time. Therefore, adolescence represents an important period in an individual's lifespan because there are considerable decreases in physical activity levels (Sallis et al., 2000; Sherar et al., 2007; Trost et al.; 2002) and, simultaneously, substantial changes in body composition.

6.3.4 Bone Length

As previously mentioned, the primary mechanical challenges to bone's mechanostat during growth comes from increases in bone length and muscle force (Frost, 1987; Rauch and Schöenau, 2001). Longitudinal growth increases lever arms and bending moments that are actuated by muscle forces. Measuring limb length has been suggested as a way to take into consideration lever arm length (Petite et al., 2005) and has begun to be used as a controlling variable in muscle-bone unit analyses (Frank et al., 2010; Macdonald et al., 2006). We did not include forearm length as a predictive variable in our models for a number of reasons. Firstly, SOS was measured at the distal radius, and radial length was not found to be a significant predictor in muscle-bone strength interactions in older adults at the distal radius (Frank et al., 2010). Secondly, the position in which grip strength was performed removed the contribution of a lever arm on muscle strength measurements. Grip strength was performed by squeezing a hand-held dynamometer with the elbow fully extended and no rotation at the wrist. This position would reduce the influence of a lever arm and its bending forces by removing the angle of rotation, in-turn minimizing the effect forearm length could have had on the amount of force generated on the radius. Taking into account the influence of lever arms in muscle-bone

unit interactions may depend on the type and location of bone being assessed along with how muscle strength and function is estimated.

6.4 Limitations

A major limitation of this research was that the physical activity, dietary and bone resorption variables included in the cross-sectional study were not included in the longitudinal analysis. This omission did not allow for a proper comparison of the full functional model of bone development between both types of study designs. On the other hand, we made the decision not to include physical activity in the empirical analysis because it was not found to be a significant predictor of radial SOS in the cross-sectional study. This was somewhat expected as the radius was specifically investigated in order to remove any influence of physical activity in the form of ground reaction forces. The disadvantage of not including physical activity in the model is our inability to discuss any potential systemic effects physical activity may have had on the functional muscle-bone unit.

Although it is a strength that we used accelerometry to measure activity levels, we only measured counts along the vertical axis which would encompass predominantly weight-bearing and ground reaction types of forces, which are loading activities that we were trying to avoid by examining the radial bone in the first place. By only recording vertical accelerations, the influences of other types of activities, such as upper body activities, that may have occurred along different planes are omitted, thereby underestimating the level of habitual physical activity in our population. In other words, the fact that not all participants wore the accelerometers for the correct number of hours and for the appropriate number of days, may have resulted in lost activity data points for those individuals.

Nutritional components were not included in the longitudinal analysis as the main purpose of the study was to examine the role of maturation on the muscle-bone unit and its direct and indirect influence on muscle and bone development. Furthermore, based on the cross-sectional analysis we did not find nutrition to act as a significant predictor of radial SOS, and it was not until NTX was included in the final model that calcium's influence became apparent. Since NTX was not part of the longitudinal analysis it was decided to not include calcium, let alone any other nutritional component, in the empirical analysis.

With respect to nutrition, 24-hour recall was used to assess dietary intake, which may not be the most realistic reflection of dietary habits in children and adolescents since it based on only one day. Similarly, dietary recall may not be the most accurate measure of intake levels for bone and muscle appropriate nutrients and can lead to incorrect and underreporting of quantities of food. We attempted to avoid this issues by prompting participants by using images of portion sizes and when possible interviewing them, particularly the younger children, alongside their guardians who often were the ones preparing their food.

Lastly, as only urine samples were collected from participants, and not blood samples, markers of bone formation and hormonal levels were not assessed. Thus, we cannot make conclusions regarding the contribution of osteoblast activity into the model, nor the contributions estrogens and androgens and other hormones (such as IGF-1) are known to have on bone and muscle properties in both boys and girls (Grumbach, 2000). In addition, it would be better to use more than one marker to monitor longitudinal growth and bone mineral accrual considering that sensitivities and predictive values of single markers are still poor and influenced by several limitations, such as diurnal variation (showing peak concentrations in the morning). It would have also been advantageous to follow a greater number of participants

longitudinally later into adolescence in order to better examine some of the already observable sex-dependent maturational effects on the muscle-bone unit. Unfortunately, our sample size was also greatly reduced for participants with a third observation point making longitudinal inferences and the inclusion of other aforementioned variables into the model more difficult.

Cross-sectionally, grip strength was corrected for anthropometrically measured forearm CSA, which took into account not only MCSA, but also bone. It is recommended that an imaging technique such as ultrasound or pQCT be used to better estimate forearm MCSA. Grip strength was not corrected for muscle size in the longitudinal study. Correcting for muscle size may have alluded to potential neuromuscular factors that contribute to muscle force over and beyond any size contributions during growth.

6.5 Strengths

Despite the aforementioned limitations, both observational studies were the first studies of the functional model of bone development in a large cohort of healthy children to include simple and relevant measures of muscle strength and bone properties, and from a cross-sectional and longitudinal perspective. Moreover, the cross-sectional study is the first detailed study of the functional model of bone development that includes the various modulators described in the model, and most importantly, markers of bone resorption. It can be argued that QUS does not present discrete information on bone content, size, geometry or strength, however, the SOS score provided does reflect both the qualitative and quantitative properties of bone that contribute to bone strength (Baroncelli, 2008). Importantly, QUS is a simple and radiation free method of bone assessment which is particularly helpful when conducting serial and multiple measures in children. A significant strength in both studies is that the muscle and

bone measures are size-independent. Therefore, size of any kind is not a confounding factor in our muscle-bone interactions, which is particularly important when investigating growing children because it allows us to emphasize that muscle strength is a primary contributor to the development of radial bone strength,

Another strength comes from the longitudinal empirical analysis that was implemented to represent the functional model of bone development and the proposed physiological effects of muscle force on bone during maturation. To our knowledge, our longitudinal study is the first study to use mediation analysis to characterize a temporal sequence of maturation, muscle force and bone strength *longitudinally*. The use of a mediated approach helps to determine where maturation plays a role in the functional model of bone development and its potential influence the muscle-bone unit. The functional model of bone development (Rauch and Schöenau, 2001) is a complex model with multiple direct and indirect pathways. Therefore, there is a need for mediated types of analysis to further elucidate the ways different variables can influence bone strength during growth.

Finally, whether cross-sectionally or longitudinally, being able to identify these relationships using less advanced techniques, not only demonstrates the strength of the proposed model of bone development, but also that QUS and simple measures of muscle force such as grip strength are effective at examining the functional muscle-bone unit in children.

6.6 Overall Conclusions

From the systematic review first we were able to determine that there may be a window of opportunity for bone response, with the early pubertal period being the best time to generate skeletal adaptations to physical activity. Secondly, the general consensus is that jumping

exercises stimulate bone the best, and may be the best modality to promoting positive changes in bone parameters, particularly in short periods of time. Thirdly, WBPAs require longer durations to see positive results, but have the ability to provide long lasting positive effects that can reduce the risk of fractures (Detter et al., 2013; Fritz et al., 2016a, 2016b; Lofgren et al., 2011, 2012).

It has been suggested that the critical property for bone health is bone strength rather than bone mass, and that the development of bone strength occurs through appropriately applied mechanical loads on bone, mainly through muscle contractions (Rauch et al., 2004; Schöenau and Frost, 2002). In particular, the concept of a muscle-bone unit challenges the notion of peak bone mass by suggesting that bone mass and strength should not be related to age, but rather to muscle strength and function (Schöenau & Fricke, 2008). The second part of this dissertation provides support for the functional model of bone development from both a cross-sectional and longitudinal perspective. Specifically, Part 2 of this dissertation was able to show that: a) QUS was effective in reflecting changes in the muscle-bone unit relationship during maturation; b) grip strength was one of the most significant predictors of radial SOS over and beyond maturity, with grip strength acting as a strong mediator in the relationship between maturation and radial SOS, particularly in girls; c) physical activity did not act as a significant independent predictor of radial SOS, as expected based on our study approach; d) NTX had a role in the regulatory feedback loop of the functional model of bone development by being a strong determinant of radial SOS, as well as a potential mediator in the effects of dietary calcium; and e) there were sex-specific differences in the muscle-bone unit relationship, with radial SOS being potentially more responsive to muscle function in girls compared to boys. These results may reflect the sex-related differences in the development of cortical bone

and may help to underscore the role muscle function has on bone strength due to the differences in cortical bone deposition between sexes during puberty.

Together, these studies provide a "big picture" view of the development of the muscle-bone unit during maturation in boys and girls, and demonstrate that radial bone strength is appropriately adapted to both muscle function and force. Overall, our methodology and findings have clinical importance because the radius is a common fracture site in youth (Khosla et al., 2003). Evaluating the functional muscle-bone unit from this perspective may increase the sensitivity of fracture prediction and how bone health is defined in this population.

6.7 Future Directions and Recommendations

The majority of strength improvements found in children are the result of neuromuscular changes with growth and maturation. However, very little is known about the neuromuscular changes that go along with these changes in muscle strength and whether they have any effects on bone strength. Future studies examining the neuromuscular components of muscle strength changes, such as peak rate of torque development, may provide further insight into the effects muscle function has on the development of bone strength.

Moreover, future studies should also investigate the functional muscle bone unit from more of a dynamic perspective by including markers of both formation and resorption. This inclusion will elucidate the actions of osteoblasts and osteoclasts on bone strength and needs to be conducted concurrently with static measures of bone strength, while including moderating variables in order to provide a better "big picture" representation of the functional model of bone development during growth. Along these lines, it can be argued that an additional pathway be added to the proposed model by Rauch and Schöenau (2001) connecting muscle

force challenges to the cellular regulatory feedback loop of bone strength. Measuring myokines from muscle cells in response to muscle contractions and, in turn, the influence of myokines on biochemical markers of bone turnover would represent the cellular component of the functional muscle-bone. This type of research has predominantly been conducted in animal models, with very little, if any, being conducted in children and adolescents.

Since the functional model of bone development is a complex model with multiple direct and indirect pathways, it is recommended that analyses go beyond the traditional total effects and complex hierarchal models to include more mediated types of analyses. These analyses would better explore how particular physiological or behavioural factors impart their influence on bone strength during growth and maturation. The two observational studies in this dissertation highlight how different types of analyses can lead to somewhat different results. Furthermore, since both a cross-sectional and longitudinal design was employed by this dissertation to investigate the functional model of bone development, the next logical step would be to conduct a study investigating the muscle-bone unit using an intervention specifically targeting only muscle strength and function adaptations in youth.

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